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Research Article

Study on Biochemical Perspectives of Antioxidant and Oxidant Indices in Oral Squamous Cell Carcinomas (OSCCs) and Other Oral Potentially Malignant Disorders (OPMDs)

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ABSTRACT:

Background-Oxidative stress in biological systems is a complex process that is characterized by an inequity between the production of free radicals (FR) and the ability of the body to eliminate these reactive species through the use of endogenous and exogenous antioxidants. The pathogenesis of oral cancer has been linked to alterations in the antioxidant defense mechanism.

Materials & Methods- Saliva from twenty patients with OSCC, forty patients with OPMDs and twenty healthy subjects in the age group of thirty five to seventy five years was analyzed for levels of nitric oxide, vitamin C, total sialic acid and GSH using spectrophotometry.

Results - The levels of salivary vitamin C and glutathione were significantly reduced and those of nitric oxide and sialic acid were raised in patients having OPMD's and oral squamous cell carcinoma. The correlation between the AOI and calculated ratios indicated that antioxidant potential of the saliva was decreased and was statistically related to ($p < 0.001$) development of OPMD, which further may progress to oral cancer, notably OSCC.

Conclusion- The current study demonstrated that the estimation of vitamin C, nitric oxide, sialic acid and GSH in saliva could be used as an early potential diagnostic biomarker in the screening of oral cancer. The antioxidant-oxidant indices (AOI's) can be used as a reliable tool for predicting the oral microenvironment and its predicted change towards development of oral cancer. This optimized developed protocol was also found to be simple and cost effective.

KEYWORDS: oral cancer; saliva; AOI; spectrophotometry

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INTRODUCTION:

Oral cancer includes a group of neoplasms affecting any region of the oral cavity, pharyngeal regions and salivary glands. Head and neck cancers (HNCs) have emerged as a leading cause of cancer-related mortality and morbidity worldwide. It is estimated that more of 90% of all oral neoplasms are OSCC.^[1] Saliva is one of the vital fluids secreted in

human beings. It is of importance to understand that the use of saliva is and will be a most promising detection method for the diagnosis of oral cancer. Obtaining saliva samples is non-invasive; there is a lower risk of infection, while direct contact of saliva with oral pathologies can contribute to the earlier detection of relevant diseases. Although the oral cavity is frequently examined, 60% of intra oral carcinomas

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are in advanced stage at the time of detection. Persistent difficulties arising in oral cancer are late diagnosis, poor response of tumor to chemotherapy, lack of reliable biomarkers for early diagnosis and post-therapeutic monitoring.^[2]

Biomarkers of oxidative stress have been a major source of debate related to monitoring oxidative stress and the possible resulting damage. Biomarkers of oxidative stress have been measured in plasma, whole blood, urine, resired gases, muscle, and other skeletal tissues. Saliva is an attractive bio-specimen for a number of reasons including the ease of its collection and the copious amount the human body is capable of producing for examination. Saliva is increasingly used and well validated in diagnosing. Moreover, saliva is reported to be suitable to detect the body's oxidative stress level.^[3]

The present study was done as a part of biochemical evaluation using standardized materials and methods to assess the antioxidant–oxidant indices (AOI) in patients having oral potentially malignant disorders and oral cancer. This was a research-based study with non-invasive laboratory experimentation on patients suffering with OSCC and OPMD with those of normal controls. The study was aimed to evaluate and compare the salivary levels of nitric oxide, vitamin C, total Sialic acid and GSH in patients with oral potentially malignant disorders (OPMDs) and oral squamous cell carcinoma (OSCC) with healthy controls. We determined the antioxidant to oxidant indices (AOI) of above mentioned biomarkers in saliva of OPMD and those of OSCC patients. Lastly, we correlated changes in AOI of vitamin C, nitric oxide, Glutathione (GSH) and sialic acid in saliva of patients with OPMD with OSCC and control patients.

MATERIALS & METHODS:

The data was collected non-invasively from patients visiting the Jawaharlal Nehru cancer center, Bhopal and Dept. of Oral Medicine and Radiology, RKDF Dental College and Research Centre, Bhopal. Informed consent was obtained from all the patients before collection of samples. The study was performed in the Department of Biochemistry, RKDF Dental College and Research Centre, Bhopal after obtaining approval from the university ethical committee.

Method Of Collection of Data - Saliva from twenty patients with OSCC, forty patients with OPMDs and twenty healthy subjects in the age group of thirty five to seventy five years was

analyzed for levels of nitric oxide, vitamin C, total sialic acid and GSH using spectrophotometry.

- a. **Study group 1:** Patients who were histopathologically diagnosed with OSCC (n=20)
- b. **Study group 2:** Patients who were histopathologically diagnosed with OPMD (n=40)
- c. **Control group:** Normal healthy individuals with clinically normal oral mucosa (n=20).

All the patients were examined using the mouth mirrors and probes under the artificial light. Their history and clinical findings were recorded using the standard proforma after informed consents.

Inclusion criteria-

1. Histopathologically diagnosed new cases of OSCC & OPMD were included in the study group.

Exclusion criteria-

1. Patients undergoing treatment such as chemotherapy and radiotherapy;
2. Past history of any major illness such as liver disease, tuberculosis, diabetes and hypertension;
3. Any history of malignancy other than oral cancer;
4. Recurrent or secondary lesions;
5. Subjects on antioxidants/multivitamin preparations;
6. The healthy controls had no habit of tobacco, alcohol, were devoid of any chronic illness and were not on any long-term medication.

Armamentarium-

- Dental oral mirror (odontoscope)
- Straight dental probe
- Plain test tubes
- Normal Saline
- Sterile container for saliva
- Cooling centrifuge
- Micropipettes with plastic disposable pipette tips
- Cuvettes
- Water bath
- Distilled water
- Beaker
- Stirrer
- Measuring cylinder
- Test tube stand
- Reagents for vitamin C, NO, GSH & Sialic acid.
- Spectrophotometer
- Auto analyzer

Sample collection and processing of saliva:

5ml of unstimulated salivary sample was collected from each patient after rinsing and spitting with normal saline (0.9% v/v) for a period of one minute between 10 am to 12 pm to avoid circadian variations. Two milliliters of saliva was collected and transferred for biochemical analysis.

Patients were given detailed information about the collection protocol:

- (a) Refrain from eating or drinking at least 90 minutes prior to salivary collection.
- (b) To sit in a comfortable position with eyes open and head tilted slightly forward.
- (c) Avoid swallowing and oral movements during collection.

Saliva samples were immediately centrifuged (1000g for 10 minutes) at 4°C to remove cell debris. The resulting supernatants were immediately transferred to 4 separate aliquots. The sample was finally centrifuged for about 15 minutes at 16,000 rpm for 5 minutes to remove the cellular components

- i. First group of aliquots were used for estimating vitamin C
- ii. Second group of aliquots were used for estimating nitric oxide
- iii. Third group of aliquots were used for estimating sialic acid
- iv. Fourth group of aliquots were used for estimating glutathione peroxidase.

Antioxidant-Oxidant Index (AOI) was calculated using the ratio between the levels of nitric oxide (NO), vitamin C, total sialic acid and GSH peroxidation levels. Post hoc Bonferroni's test analysis was used for the comparison of the two study groups to the control group. The data is expressed as mean \pm SD. The statistical significance of the results was analyzed using post hoc Bonferroni's test. Correlation between the groups was done using Pearson's correlation coefficient test.

RESULTS:

The study comprised of 80 subjects, there were 27 females (34%) and 53 males (66%) in the study group. There were fifty-three males (controls-10; OPMD-30; OSCC-13) and twenty-seven females (controls-10; OPMD-10; OSCC - 07). The mean age was 36.4 ± 1.58 years in control group, 39.30 ± 10.57 years in OPMDs and 49.25 ± 15.58 years in OSCC.

Out of 40 OPMD patients, 5 (12.5%) were without any habit, 18 (45%) with tobacco chewing, 10 (25%) with smoking and 7 (17.5%) with both tobacco chewing and smoking habits.

Among 20 OSCC patients, 1 (5%) was without

any habit, 6 (30%) with habit of tobacco chewing, 4 (20%) with smoking and 9 (45%) with both smoking and tobacco chewing.

Our results showed that, among 40 OPMD patients, leaukoplakia was seen in 25 (62.5%) cases, followed by oral submucous fibrosis in 9 cases (22.5%) and lichen planus in 6 (15%) cases.

7 (35%) cases revealed buccal mucosal lesions, 6 (30%) patients showed tongue lesions, 3 (15%) cases showed lesions on tonsil, 2 (10%) showed lesions involving alveolus. Palatal and retromolar trigone had 1 (5%) case each.

Descriptive Statistics of Variables-

The data was recorded and analyzed statistically using SPSS software version 20.0 using one way ANOVA and post hoc Bonferroni's tests. Mean of controls and patients were compared using Student's t-test. The difference was considered statistically significant when *p*-value were 0.001 or less.

The mean salivary vitamin C level was 30.32 ± 4.34 $\mu\text{mol/l}$ in OSCC group whereas; it was 38.20 ± 8.45 $\mu\text{mol/l}$ in OPMDs group and 48.76 ± 2.60 $\mu\text{mol/l}$ in control group.

The mean nitric oxide level was 27.34 ± 5.51 $\mu\text{mol/l}$ in OSCC group, 22.5 ± 2.33 $\mu\text{mol/l}$ in OPMD group and 10.11 ± 0.88 $\mu\text{mol/l}$ in control group.

The glutathione reductase activity in control patients was found to be 0.0915 U/ml under optimal pH, temperature and K_m . In OPMD group the GR activity was found to be 0.0515 U/ml. Similarly, the activity in the OSCC group was found to be 0.0292 U/ml.

The total sialic acid (TSA) in the saliva of control patients was found to be 41.241 ± 5.3312 $\mu\text{g/mL}$. In the case of OPMD patients it was 64.25 ± 4.33 $\mu\text{g/mL}$ and in the OSCC patients it was, 79.60 ± 6.93 $\mu\text{g/mL}$. As seen from the graphs above, the levels of salivary vitamin C and glutathione were significantly reduced [Graph 1 & Graph 2] and those of nitric oxide and sialic acid were raised [Graph 3 & Graph 4] in patients having OPMD's and oral squamous cell carcinoma. The antioxidant to oxidant index (AOI) was measured between NO and vitamin C; NO and GSH; total sialic acid and vitamin C and lastly, total sialic acid and GSH. The following indices are tabulated below with the corresponding values. (Tables 1,2,3,4).

It can be seen from the analysis that there was a statistically significant difference between the reduced levels of vitamin C and GSH to those that of raised nitric oxide and sialic acid levels in patients

Table 1: AOI in controls and study groups. *ANOVA, *p*-value <0.001 considered statistically significant

variable	group	n	mean	Std. Deviation	<i>p</i> -value
AOI NO / VIT.C	Control	20	0.207	0.023	
	OPMD	40	0.589	0.166	<0.001
	Cancer	20	0.901	0.279	

Table 2: AOI in controls and study groups. *ANOVA, *p*-value <0.001 considered statistically significant.

variable	group	n	mean	Std. Deviation	<i>p</i> -value
AOI NO / GSH	Control	20	0.11	0.012	
	OPMD	40	0.436	0.122	<0.001
	Cancer	20	0.936	0.289	

Table 3: AOI in controls and study groups. *ANOVA, *p*-value <0.001 considered statistically significant.

variable	group	n	mean	Std. Deviation	<i>p</i> -value
AOI TSA / VIT.C	Control	20	0.845	0.093	
	OPMD	40	1.687	0.466	<0.001
	Cancer	20	2.625	0.812	

Table 4: AOI in controls and study groups. *ANOVA, *p*-value <0.001 considered statistically significant

variable	group	n	mean	Std. Deviation	<i>p</i> -value
AOI TSA / GSH	Control	20	0.45	0.052	
	OPMD	40	1.247	0.351	<0.001
	Cancer	20	2.72	0.842	

Table 5: Pairwise comparison of AOI index in control and study groups. *Post-hoc Bonferroni's analysis with ANOVA, *p*-value <0.001 considered statistically significant.

Dependent variable	(i) group	(J) group	mean Difference (i-J)	<i>p</i> -value
AOI NO / VIT.C	Control	OPMD	-0.382*	0.001
		Cancer	-0.694*	0.001
	OPMD	Control	0.382*	0.001
		Cancer	-0.312*	0.001
		Control	0.694*	0.001

Table 6: Pairwise comparison of AOI index in control and study groups. *Post-hoc Bonferroni's analysis with ANOVA, *p*-value <0.001 considered statistically significant.

Dependent variable	(i) group	(J) group	mean Difference (i-J)	<i>p</i> -value
AOI NO / GSH	Control	OPMD	-0.326*	0.001
		Cancer	-0.826*	0.001
	OPMD	Control	0.326*	0.001
		Cancer	-0.500*	0.001
		Control	0.826*	0.001

having or suffering from OPMD's and those having histopathologically proven cancer of oral cavity. The data where *p*-value <0.001 is statistically significant.

Post – hoc Bonferroni's analysis was performed for pairwise comparison between the controls, OPMD and OSCC patients. The analysis clearly indicates

Table 7: Pairwise comparison of AOI index in control and study groups. *Post-hoc Bonferroni's analysis with ANOVA, *p*-value <0.001 considered statistically significant.

Dependent variable	(i) group	(J) group	mean Difference (i-J)	<i>p</i> -value
AOI	Control	OPMD	-0.842*	0.001
		Cancer	-1.420*	0.001
	OPMD	Control	0.842*	0.001
		Cancer	-0.938*	0.001
TSA / VIT.C	Cancer	Control	1.420*	0.001

Table 8: Pairwise comparison of AOI index in control and study groups. *Post-hoc Bonferroni's analysis with ANOVA, *p*-value <0.001 considered statistically significant.

Dependent variable	(i) group	(J) group	mean Difference (i-J)	<i>p</i> -value
AOI	Control	OPMD	-0.797*	0.001
		Cancer	-2.270*	0.001
	OPMD	Control	0.797*	0.001
		Cancer	-1.473*	0.001
TSA / GSH	Cancer	Control	2.270*	0.001

Table 9: *p*-value significance chart between OPMD and OSCC group

Variables	(A) Group	(B) Group	Mean Difference (A-B)	Standard error	<i>p</i> -value
VITAMIN C	Control	OPMD	10.56	2.489	0.003
	Control	OSCC	18.44	2.489	0.003
	OPMD	OSCC	7.88	1.889	0.001
NITRIC OXIDE (NO)	Control	OPMD	-12.39	1.81	0.001
	Control	OSCC	-17.23	1.81	0.001
	OPMD	OSCC	-4.44	1.344	0.001
GLUTATHIONE	Control	OPMD	0.04	0.0177	0.003
	Control	OSCC	0.0623	0.0177	0.003
	OPMD	OSCC	0.0223	0.0177	0.003
TOTAL SIALIC ACID	Control	OPMD	-23.009	2.389	0.001
	Control	OSCC	-38.359	2.389	0.001
	OPMD	OSCC	-15.349	1.891	0.001

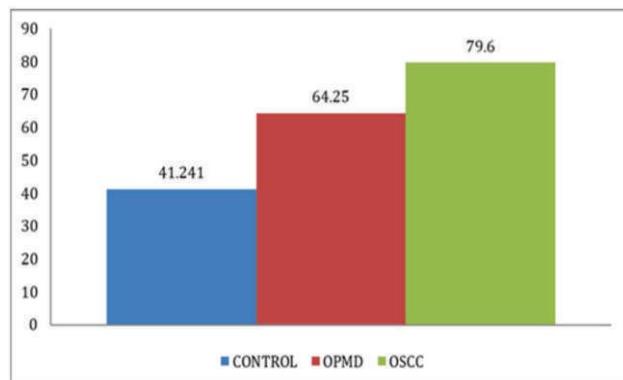
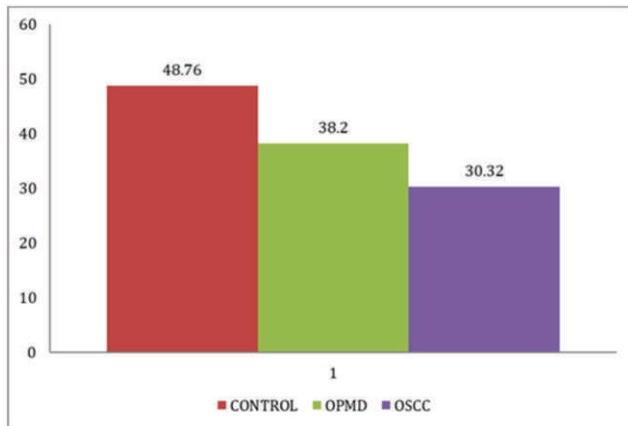
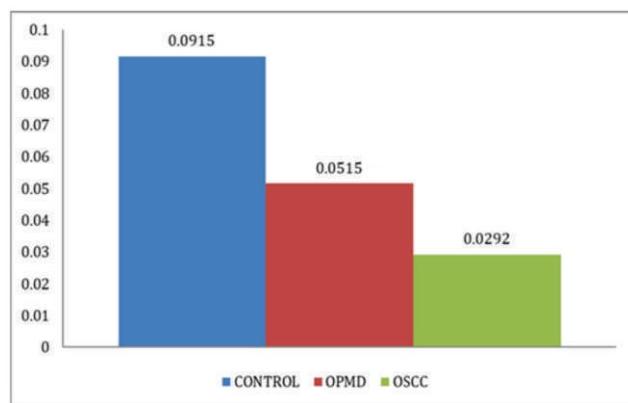
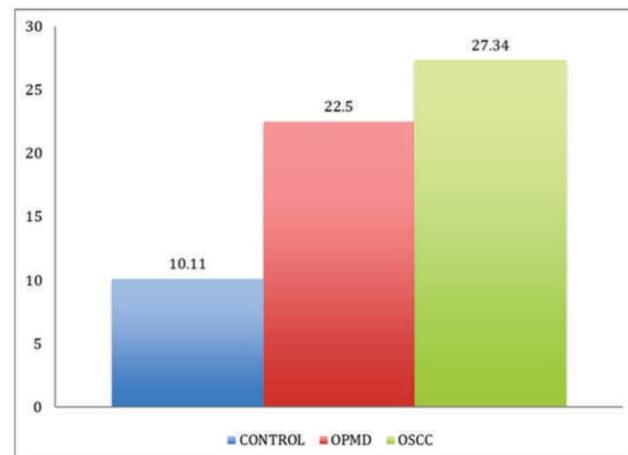
statistical difference between the co-relation between the values of raised NO levels to those of vitamin C. This significant statistical relationship where *p*-value is less than 0.001 indicates changes in the biochemical nature of saliva with patients having mucosal potentially malignant disorders as well as oral cancer. (Tables 5,6,7,8).

Our results were consistent with the grouped analysis between various salivary biomarkers as can be

from the tabulated data above. There was significant increase in AOI [NO/Vit.C] from control group (0.023), OPMDs (0.167) and OSCC group (0.279) (Table 1)

And AOI [NO/GSH] from control group (0.012), OPMDs (0.122) and OSCC group (0.289) (Table 2)

Similarly the increase was seen in AOI [TSA/Vit.C] from control group (0.093), OPMDs

**GRAPH 1:** Mean concentration of Vitamin C ($\mu\text{mol/L}$).**GRAPH 2:** Mean concentration of salivary glutathione (U/ml).**GRAPH 3:** Mean concentration of nitric oxide - NO ($\mu\text{mol/L}$).

(0.467) and OSCC group (0.812)(Table 3). The AOI [TSA/GSH] showed increase from control group (0.052), OPDs (0.351) and OSCC group (0.842)(Table 4). On comparing salivary vitamin C levels and GSH levels with those of the nitric oxide and sialic acid, the difference was highly significant ($p<0.003$). Though the levels were not significant between OPMD and OSCC group ($p>0.001$) (Table 9).

The levels of salivary vitamin C and glutathione were significantly reduced and those of nitric oxide and sialic acid were raised in patients having OPMD's and oral squamous cell carcinoma.

DISCUSSION:

A biomarker is defined as a pharmacological or physiological measurement that is used to predict a toxic event; a specific molecule in the body, which has a particular feature that makes it instrumental for measuring disease progression or the effects of treatment. Biomarkers are by definition suitable to develop new diagnostic tools, alone or in combination with traditional methods (Brinkman and Wong, 2006).^[4]

The immune system of an individual works in a very well-organized manner for the sustenance of the normal equilibrium, thus helping in achieving a disease-free state. Free radicals are generated as a by-product of normal cellular metabolism and the increase in the levels of ROS may be caused either due to increased production or decreased destruction of these formed free radicals by the enzymatic and nonenzymatic antioxidants. To control the influx of ROS, aerobic cells have developed their own defense system – the antioxidant protection system, which includes enzymatic and nonenzymatic components that function interactively and synergistically to neutralize free radicals.^[5]

Nitric oxide (NO) is a highly reactive oxygen radical found in normal and malignant tissues, however its levels are much higher in malignant tissue. Its generation is thought to be by a family of enzymes called nitric oxide synthase (NOS). NOS are available in three isoforms NOS1 or type 1 or nNOS (neuronal), NOS2 or type 2 or iNOS (inducible), NOS3 or type 3 or eNOS (endothelial). Out of these, iNOS produces continuous NO and is shown to be expressed in many malignant tumors.^[6]

Vitamin C is a major water-soluble antioxidant. Generally, vitamin C is a six carbon

organic acid with structural similarity to glucose. It acts as a potent reducing agent and its laevo (l-) form is generally more active. Vitamin C has been shown, together with some other antioxidant agents, to be an endogenous modulator of the metabolism of nitric oxide (NO) and subsequent endothelium-dependent vasodilation. The difference in NO production at the periodontal level is probably different from NO in the bloodstream: In the mouth, it is an antibacterial defense, whereas systemically, it impacts endothelial function.^[7,8]

Glutathione occurs in high concentrations (0.5 to 10mmol/L) in virtually all cells. Cellular GSH concentrations are reduced markedly in response to protein malnutrition, oxidative stress and many pathological conditions. Salivary glutathione levels may be an index of oxidative stress at the level of the upper airways and in particular of oral cavity and pharynx. Therefore, high salivary glutathione may be an epidemiological marker to identify subjects with an increased risk of developing HNSCC, to submit to strict follow-up and chemoprevention. Metabolic alterations of saliva could be both an epidemiological marker and a target for chemoprevention of oral and oropharyngeal carcinogenesis.^[9,10] Sialic acid plays a significant role in cancer due to increased sialylation and sialyltransferase activity.^[11]

In our study, levels of nitric oxide were lowest in control group but increased significantly in OPMDs and OSCC groups. The levels of nitric oxide in OPMDs and OSCC were comparable. This is in accordance with the study done by Juneja S et al^[12], who also reported increased level of nitric oxide in OPMD and OSCC patients.

Serum levels of vitamin C were highest in control group and reduced significantly in OPMDs and OSCC group in the present study. There was statistically significant difference in the levels of vitamin C in OPMDs and OSCC also. Vitamin C reduces the degradation of Vitamin E thus enhancing chemotaxis, phagocytosis and collagen synthesis. It inhibits the formation of nitrosamines and causes reduction in oncogene expression. Vitamin E maintains the integrity of membranes thus inhibiting the growth of cancer cell and differentiation. It also inhibits mutagenicity and formation of nitrosamines. Synergistic action between Vitamin E, selenium and ascorbate hinders DNA and RNA protein synthesis in the cells.^[13]

Glutathione participates in detoxification at several different levels, and may scavenge free radicals, reduce peroxides or be conjugated with electrophilic compounds. Thus, glutathione provides

the cell with multiple defences not only against ROS but also against their toxic products. In the study done by us, the levels of salivary glutathione reductase were lower in OSCC when compared to OPMD and the difference was statistically significant ($p<0.003$)

In our study, the total sialic acid (TSA) in the saliva of control patients was found to be $41.241\pm 5.3312 \mu\text{g/mL}$. In the case of OPMD patients it was $64.25 \pm 4.33 \mu\text{g/mL}$ and in the OSCC patients it was, $79.60 \pm 6.93 \mu\text{g/mL}$. We also found significantly higher levels of free sialic acid in well-differentiated OSCC patients compared to those of moderately differentiated cases. This suggests correlation of elevated salivary sialic acid levels to the progression of OSCC. There is elevated salivary sialic acid level in moderately /poorly-differentiated squamous cell carcinoma without any significant change in well-differentiated squamous cell carcinoma. This finding was consistent with the study by Rajaram S et al.^[14]

CONCLUSION:

The findings of the present study indicate that estimation of Vitamin C, nitric oxide, GSH and sialic acid can be suitably used and could assist in the early diagnosis of potentially malignant disorders and oral cancer using saliva. OSCC increases oxidative stress and may trigger mutations, suggesting that it may play a role in the initiation and development of multistage carcinogenesis. Understanding the function of reactive oxygen species (ROS) as key mediators in signaling pathways may open up new avenues for pharmacological intervention.

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Conflicts of interest

There are no conflicts of interest.

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Research Article

Oral Submucous Fibrosis: Clinical Stage, Functional Stage and Histopathological Grade Correlation

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ABSTRACT:

Background- Oral submucosal fibrosis (OSMF) is a common precancerous condition predominantly affecting the oral cavity. There are several contributing factors to its pathogenesis, areca nut chewing being the primary culprit. OSMF reversal is difficult once the disease sets in. Hence, after its diagnosis, staging becomes imperative as it affects the treatment plan. Several classification methods have been developed by various authors to categorize the disease based on its clinical, functional, and Histopathological characteristics. The main aim to conduct this study was to correlate clinical and histopathological staging.

Materials & Methods- This study involved 30 patients who had been diagnosed with OSMF both Clinically and Histopathologically. All 30 patients were classified and were categorized according to their clinical and functional stages after inspection. Following this, histopathological grading was done.

Results- The statistical analysis showed that there was a wide similarity between the clinical and functional staging. However, no significant similarity or correlation was found between the clinical and functional staging with its corresponding histopathological grades.

Conclusions- Our study concluded that there is strong correlation between clinical and functional grading.

KEYWORDS: clinical staging; functional staging; histopathological grading; oral submucous fibrosis

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INTRODUCTION:

Oral submucous fibrosis (OSMF) is a precancerous condition that affects the oral cavity^[1] and was first mentioned by Schwartz in 1952 under the term “atrophica idiopathica mucosae oris”^[2]. It is a slow-developing, chronic disease characterized by inflammation and fibrosis in the submucosal tissues.^[3] Joshi later coined the term “OSMF” in 1953.^[4] Studies have confirmed that areca nut is the main cause of OSMF,^[5-8] although other factors such as deficiencies in

iron, zinc, essential vitamins, and capsaicin in chilies, may also play a role.^[9-12] Various classification systems have been put forth by different authors, which categorize patients based on their clinical signs and symptoms, functional abilities and histopathological findings.^[13] The main purpose of the classification system is to identify this premalignant disorder in its early stage and to formulate a treatment plan accordingly for patient's speedy recovery.^{[14],[15]} This

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study aims to evaluate the agreement and correlation between the clinical staging, functional staging and histopathological grading of OSMF patients.

MATERIALS & METHODS:

The present study was carried out in the Department of Oral Pathology and Microbiology at Bhabha College of Dental Science in Bhopal, India. The research ethics committee of the institute approved the study and informed consent was obtained from each patient. The study population was composed of patients visiting the Outpatient Department of Oral Pathology and Microbiology. The study group consisted of 30 individuals diagnosed with OSMF both clinically and histopathologically. Participants were seated comfortably in a dental chair for examination and data was recorded using a standardized form. The clinical examination followed the method outlined by Kerr, Ash, and Millard. Interincisal mouth opening was measured using a divider and scale and recorded in millimeters. Local anesthesia was administered, an incisional biopsy was taken from the area with palpable fibrous bands, and the specimens were preserved in 10% formalin for further laboratory procedures. Post-surgical instructions were given and sutures were placed, with cotton kept at the biopsy site.

The functional and histopathological staging of OSMF was done according to Khanna J N and Andrade N N.^[16] Clinically subjects were grouped into four categories, according to functional staging and Histological staging was divided into four groups, Group I, Group II, Group III and Group IV.

Statistical Analysis-

Following online calculators were used for statistical analysis of the data.

Kappa (k)	www.graphpad.com
Spearman's r	www.gigacalculator.com
Pearson Chi-square test	www.atozmath.com
<i>P</i> -value	www.socscistatistics.com

RESULTS:

The present study was undertaken to assess the correlation between clinical staging, functional staging, and histopathological grading of patients with OSMF. A total of 30 patients were included in the study with an age range between 11-60 years, with a mean of 36 years.

The youngest patient was 17 years old, and the only female patient was 20 years. Most of the subjects 23 (76.6%) were in the age range of 21-50 years [Table 1].

Table 1: Distribution of cases with respect to Age and Gender.

Age group	Male	Female	Number of patients
11-20	1	1	2
21-30	7	0	7
31-40	6	2	8
41-50	6	2	8
51-60	4	0	4
Total	25	5	30

A total of 30 patients were graded according to functional and clinical criteria. Out of these at least in 25 patients, the functional grading was similar to the clinical staging [Table 2]. Hence, the percentage of agreement was 83.3%. No agreement or poor agreement was seen in 5 cases. Overall, there was a good agreement between clinical and functional grading.

Out of 30 patients, only six patients presented clinical staging similar to histopathological grading [Table 3]. Twenty-four patients presented with no agreement or poor agreement. Hence, the percentage of agreement was 20%. There was a poor agreement between clinical and histopathological grading.

There was no significant correlation between clinical and histopathological grading.

Out of 30 patients, functional grading was similar to histopathological staging in only 7 patients [Table 4]. No agreement or poor agreement was seen in 23 patients. The percentage of agreement was only 23.3%. There was a poor agreement between functional and histopathological grading.

There was no significant correlation between functional and histopathological grading.

DISCUSSION:

The purpose of this study was to investigate the relationship between the clinical staging, functional grading, and histopathological grading of patients with OSMF. 30 patients participated in the study, 25 of whom were male and 5 were female, a demographic that aligns with previous findings of a higher male predilection for OSMF in the literature. Most of the patients were of age ranging between 20 to

Table 2: Clinical and functional staging assigned to the patients.

Functional grading	Number of patients with clinical stage 1	Number of patients with clinical stage 2	Number of patients with clinical stage 3	Number of patients with clinical stage 4	Number of patients (%)
Stage 1	5	1	0	0	6 (20)
Stage 2	2	13	1	0	16 (53.3)
Stage 3	1	0	6	0	7 (23.3)
Stage 4	0	0	0	1	1 (3.3)
Total, n (%)	8 (26.7)	14 (46.6)	7 (23.3)	1 (3.3)	30

Test for agreement	Agreement
Measure of Agreement	
Weighted kappa (k)	$k = 0.755$ (Substantial agreement)
Correlation coefficient	
Spearman's coefficient	$r = 0.8$ ($p < 0.001$; significant)

Table 3: Clinical and Histopathological staging assigned to the patient.

Histopathological Grading	Number of patients with clinical stage 1	Number of patients with clinical stage 2	Number of patients with clinical stage 3	Number of patients with clinical stage 4	Number of patients (%)
Grade 1	1	2	0	0	3 (10)
Grade 2	3	2	4	0	9 (30)
Grade 3	2	5	3	1	11 (36.6)
Grade 4	2	5	0	0	7 (23.3)
Total, n (%)	8 (26.7)	14 (46.6)	7 (23.3)	1 (3.3)	30

TEST FOR AGREEMENT	AGREEMENT
Measure of agreement	
Weighted kappa (k)	$k = -0.050$ (No agreement)
Correlation coefficient	
Spearman's coefficient	$r = 0.15$ ($p = 0.4662$; No Correlation)

50 years, a finding that is consistent with previous studies.

All patients in the study reported a positive history of chewing raw areca nut, which is known to be a major cause of OSMF. The most commonly used form of areca nut was found to be Gutkha, with 66.6 % of patients consuming it. The use of areca nut was more prevalent in females, while the use of Gutkha was more prevalent in males.

Commercial freeze-dried betel quid substitutes (such as Pan Masala, Gutkha, and Mawa), conveniently packaged in portable sachets, have become increasingly popular because they have a long shelf life and do not require preparation before use. These products contain a higher concentration of areca nut and appear to cause oral submucous fibrosis more rapidly than conventionally prepared betel quid^[17].

Table 4: Functional and Histopathological Staging assigned to the patients.

Histopathological	Number of patients with Functional stage 1	Number of patients with Functional stage 2	Number of patients with Functional stage 3	Number of patients with Functional stage 4	Number of patients (%)
Stage 1	1	1	1	0	3 (10)
Stage 2	3	3	3	0	9 (30)
Stage 3	3	4	3	1	11 (36.6)
Stage 4	3	3	1	0	7 (23.3)
Total, n (%)	10 (33.3)	11 (36.6)	8 (26.6)	1 (3.3)	30

Test for agreement	Agreement
Measure of Agreement	
Weighted kappa (k)	$k = -0.036$ (No agreement)
Correlation coefficient	
Spearman's coefficient	$r = 0$ ($p = 0.9733$; No Correlation)

All 30 patients underwent clinical staging, and 8 were classified as Stage 1, 14 as Stage 2, 7 as Stage 3, and 1 as Stage 4. The functional staging was also done for all patients, with 6 as Stage 1, 16 as Stage 2, 7 as Stage 3 and 1 as Stage 4. The histopathological examination revealed that 3 patients were included in Grade 1, 9 in Grade 2, 11 in Grade 3 and 7 in Grade 4.

Studies by Biradar et al.^[18], Pandya et al.^[19], Radhika et al.^[20], Shivakumar and Sahana^[21], Goel et al.^[22] and Bhatt et al.^[23] have concluded that there is a significant correlation between the functional and histopathological stages of OSMF, but no significant correlation between the clinical and histopathological stages. Our findings align with these previous studies and suggest that clinical signs and symptoms can affect normal functional abilities, such as mouth opening, tongue protrusion, and cheek puffing. These discrepancies should be evaluated to demonstrate the functional loss to the patient and motivate them to seek treatment.

CONCLUSION:

The objective of this study was to examine the relationship between clinical staging, functional staging, and histopathological grading of OSMF. We concluded that there may be a strong correlation between clinical and functional grading. However, the

correlation between clinical and histopathological grading or between functional and histopathological grading was non-significant. The strong correlation between clinical and functional grading provides hope that in the future, it may be possible to make predictions about the prognosis of the disorder based on these classifications.

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Conflicts of interest

There are no conflicts of interest.

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Research Article

Comparative Evaluation of the Effects of Black Tea Extract Mouthrinse and Chlorhexidine Mouthwash on Salivary Streptococcus Mutans Load

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ABSTRACT:

Background- Dental caries is one of the most frequent oral health problems. The present study shows the antibacterial effect of black tea extract on salivary *Sterptococcus Mutans* load.

Materials & Methods- The study was conducted on 125 individuals. The differences in the Colony Forming Units and count-scores of *S.mutans* were analyzed in salivary samples collected from individuals before and after administration of 2% black tea extract mouth-rinse and chlorhexidine mouthwash(CM).

Results- There was a statistical difference in mean salivary *S. mutans* colony count and mean count-score before and after administration of black tea extract mouth-rinse ($p = 0.0003$) and chlorhexidine mouthwash ($p = 0.0002$) respectively. Hence, it was found that there is no statistically significant difference in the fall of *S.mutans* load due to black tea mouth-rinse and chlorhexidine mouthwash.

Conclusions- A 2% black tea extract mouth-rinse significantly reduces salivary *S.mutans* load, irrespective of age and gender. Also, it is an effective natural anti-cariogenic agent with no known implicated side effects.

KEYWORDS: dental caries; oral health; streptococcus mutans; black tea extract mouth-rinse; chlorhexidine mouthwash.

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INTRODUCTION:

Dental caries is a public health problem throughout the world. In the western world, the prevalence of caries has declined, but 5-20% population still remain at high risk.^[1] Many factors, both local like diet, tooth structure and anatomy, saliva, plaque, crevicular fluid, bacteria and systemic like age, gender, race, religion, culture, familial and genetic

factors, socio-economic and nutritional status influence the likelihood of caries developing and its speed of progression, so that caries is truly a multifactorial disease.^[2] The association of Streptococcus mutans (*S.mutans*) and dental caries was first reported by Clarke (1924). Since then, the experiments with gnotobiotic animals have revealed mutans streptococci to be the main etiological

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microorganisms in causation of dental caries.

The *S.mutans* is a facultatively anaerobic, gram-positive coccus. The mutans streptococci comprise of a group of seven species (mutans, sobrinus, cricetus, ratus, downeii, ferus and macciae). *S.mutans* and Streptococcus sobrinus are the predominant species isolated from human saliva and dental plaque.^[3] These organisms are unique in their cariogenic potential. They are acidogenic and aciduric and once established, they can survive even in unfavourable conditions. Besides, the microorganisms in dental plaque degrade the dietary carbohydrates producing lactic acid leading to localized demineralization and the eventual formation of dental caries. *S.mutans* also encourage the accumulation and adherence of plaque biofilm by metabolizing sucrose into sticky glucan. It is now confirmed that *S. mutans* are the major bacteria responsible for the initiation of a carious lesion followed by Lactobacillus species which may be responsible for caries progression.^[4] Thus, the obvious role of *S mutans* that was reflected all the way long in the causation of dental caries, justifies the need for its elimination or reduction in order to prevent occurrence of carious lesions.

Drug resistance and side effects encountered with the use of synthetic drugs has led to the surge for novel and safe alternatives. Since ancient times, plants have proven to be an archetypal source of medicine. One such plant of high medicinal use is the black tea (*Camellia sinensis*). The leaves of this plant are usually handpicked and based on the processing of the leaves three different types of tea are produced, namely green tea (non-fermented), Oolong tea (semi-fermented) and Black tea (fermented). Black tea being the fermented type possesses more raw nutrients and health effective compounds than the other two types.^[5] Data available also enumerates some of the anticariogenic actions of certain components of black tea like bringing about remineralization of the dental hard tissues due to the release of calcium, phosphorus and fluoride ions.^[6] The purpose of this study was to analyse the effect of black tea extract mouth-rinse, a natural measure with reduced side effect, in the prevention of dental caries.

MATERIALS & METHODS:

This study was carried out with the approval of Institutional Ethical Committee and Bhabha College of Dental Sciences, Bhopal. The study was conducted in the Department of Oral Pathology & Microbiology on individuals in the age range of 21-40 years and a written informed consent for the procedure was obtained from them.

A total of 125 individuals in the age range of 21-40 years were randomly selected as per the inclusion and the exclusion criteria from those reporting to the Bhabha College of Dental Sciences, Bhopal. They were divided into three groups (a) administered Black tea extract mouth-rinse (BTEMR) in 50 subjects (b) administered Chlorhexidine mouthwash (CM) in 50 subjects and (c) administered distilled water in 25 subjects.

Inclusion Criteria-

Individuals with or without dental caries in the age group of 21-40 years.

Exclusion Criteria-

a) Completely or partially (more than 4 teeth missing) edentulous patients; b) patients who had received any antibiotic therapy in the last 14 days prior to the study; c) patients who underwent topical fluoride application in the last 3 days or mouth wash gargles within the last 12 hours prior to the study and d) patients with any known systemic disease.

BTEMR made by 2 gm of dried black tea leaves were boiled in 100 ml of tap water for 3 minutes in a stainless-steel utensil. The solution was allowed to cool for 10 minutes and then sieved into a disposable glass. This BTEMR was prepared fresh before use.

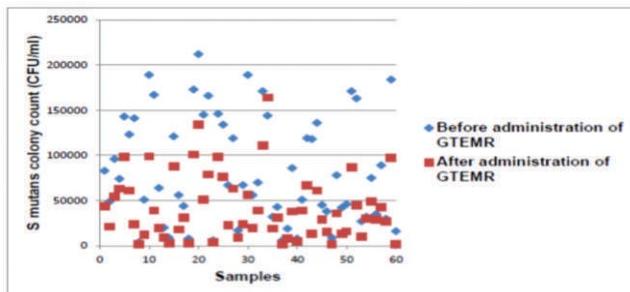
The individuals were briefly explained about the procedure prior to sample collection. They were instructed to maintain their normal oral prophylaxis and were instructed to avoid brushing or eating 1 hour prior to the saliva collection. Unstimulated saliva sample was collected from all the individuals participating in the study prior to and after the administration of BTEMR. Each individual of the study population was instructed to spit around 1-2 ml of saliva in a sterile wide mouth glass bottle. After having collected the first saliva sample, the individuals were instructed to gently rinse their mouth with 10-20 ml of the prepared BTEMR for 2 minutes and a second saliva sample was collected again after 30 minutes in another sterile glass bottle. The same procedure was followed for the other two groups, where instead of BTEMR, Chlorhexidine Mouthwash (CM) or distilled water were administered.

Culture media was prepared as follows: 90 gm of Mitis Salivarius dehydrated agar was added to 1 litre of distilled water and boiled on a Bunsen burner to dissolve completely. To this 20 gm of sucrose per 100 ml was added. This solution was then sterilized by autoclaving for 15 minutes at 15 lb per square inch at 121°C. The solution was allowed to cool to 50°C. Potassium tellurite (0.1 mg/ml), bacitracin (0.2

Table 1: Distribution and comparison of the salivary *S.mutans* colony count (CFU/ml) and the colony score in all the subjects before and after the administration of BTEMR.

		N	Mean	Standard Deviation	Difference of means	p-value
CFU/ml (x 10 ⁴)	Before	50	8.3700	5.9713	4.1134	<0.0001 (HS)
	After	50	4.2566	3.6510		
Colony Score	Before	50	1.3448	0.6695	0.1573	0.0003 (HS)
	After	50	1.1875	0.7124		

HS: Highly Statistically Significant

**Graph 1:** Distribution and comparison of the salivary *S.mutans* colony count (CFU/ml) in all the subjects before and after the administration of BTEMR.

units /ml) and human blood (2% v/v) were added to this solution. It was then well mixed and poured equally into sterile petri dishes.

0.1ml of diluted 10⁻² saliva sample was cultured on agar by spread plate technique. The plates were then incubated at 37°C for 48 hours in plastic bags inflated with expired air to enhance the carbon dioxide.

The number of colonies grown on the agar surface were counted with a magnifying glass in front of an illuminated source. Number of Colony Forming Units (CFUs) per ml of saliva was calculated as follows. CFU in 0.001 ml = 'x' (Number of colonies)

Therefore, CFU/ml = 'x' × 1000

Thus, colony forming units (CFU)/ml of saliva was calculated and scored for each saliva sample.

Scoring of salivary *S. mutans* count:

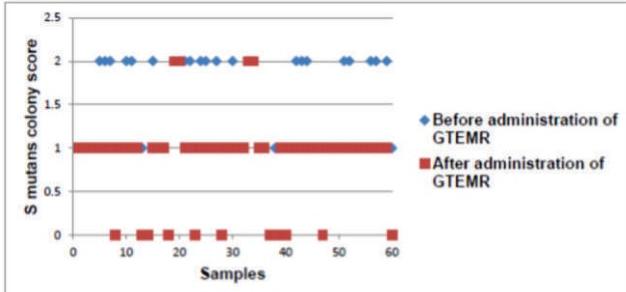
0 < 10,000 CFU/ml

1 < 100,000 CFU/ml

2 = 100,000-1,000,000 CFU/ml

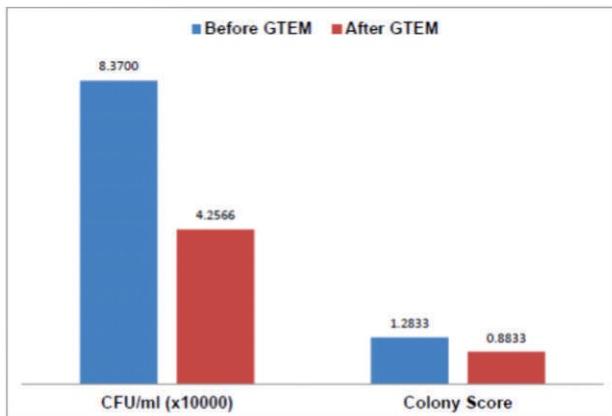
3 > 1,000,000 CFU/ml

CFU Colony Forming Unit.

**Graph 2:** Distribution and comparison of the salivary *S.mutans* colony score in all the subjects before and after the administration of BTEMR.

125 individuals in the age range of 21-40 years, divided into three groups and were administered BTEMR (n = 50), CM (n = 50), and distilled water (n = 25) Salivary samples were collected from each individual twice; the first sample which was collected prior to the administration of BTEMR, CM or distilled water, and the second sample was collected 30 min after the administration of BTEMR, CM or distilled water.

We compared *S.mutans* CFU/ml with the

**Graph 3:** Comparison of the mean salivary *S.mutans* load before and after the administration of BTEMR.

colony score before and after administration of BTEMR and found there was drastic reduction in colony score from 1.3448 to 1.1875. These results were highly significant [Table 1] [Graph 1, Graph 2 and Graph 3]. We found in our study that the results were almost similar when we compared the distribution and

STATISTICAL ANALYSIS:

Paired *t* test was applied to analyse the difference in the CFU/ml. *p*-value of <0.05 was considered significant for the differences in the mean of CFU/ml and *S. mutans* count scores before and after rinsing with BTEMR, CM or distilled water.

RESULTS:

The present study included a study group of

Table 2: Distribution and comparison of the salivary *S.mutans* colony count (CFU/ml) and the colony score in all the subjects before and after the administration of CM.

		N	Mean	Standard Deviation	Difference of means	p-value
CFU/ml (x 10 ⁴)	Before	50	7.1700	9.0713	3.2134	<0.0001 (HS)
	After	50	3.9566	4.7630		
Colony Score	Before	50	1.1432	0.6787	0.2891	0.0002 (HS)
	After	50	0.8541	0.5612		

HS: Highly Statistically Significant

Table 3 : Distribution and comparison of the salivary *S.mutans* colony count (CFU/ml) and the colony score in all the subjects before and after the administration of distilled water.

		N	Mean	Standard Deviation	Difference of means	p-value
CFU/ml (x 10 ⁴)	Before	25	8.2134	5.1533	0.6368	0.19 (NS)
	After	25	7.5766	6.8756		
Colony Score	Before	25	1.6332	0.9867	0.3978	0.21 (NS)
	After	25	1.2354	0.7586		

NS: Non Significant

colony score before and after the administration of CM and BTEMR. These results were also highly significant [Table 2]. However, when we used distilled water as the expectorant the results were not significant [Table 3].

DISCUSSION:

Among the various health issues being faced in day-to-day life, dental caries is one of the most common chronic diseases of modern times. Dental caries is associated with the frequency of fermentable carbohydrate intake. Also, certain dietary substances are more cariogenic than others. Simple sugars (e.g., Sucrose) are more cariogenic than complex sugars (e.g., Starch).^[7] Caries is initiated by *S.mutans*, whereas Lactobacilli help further progression of the lesion inside the dentin.^[8] Sakeenabi and Hiremath, Ravindran et al. and Pannu et al, concluded that *S.mutans* are the major pathogens responsible for dental caries.^[9-12]

The microflora in dental caries is highly complex and varies between individual lesions. Mutans group Streptococci, such as *S.mutans* and *Streptococcus sobrinus*, and Lactobacilli are important in the initiation and progression of caries. These microorganisms are acidogenic (produce acid) by fermenting dietary carbohydrates, which result in the demineralization of enamel and dentin. They are also aciduric (acid tolerant), which gives them a competitive survival advantage.^[8]

S.mutans and lactobacilli are regarded as the two chief bacterial species, responsible for decay in teeth. The relationship between oral cariogenic *S.mutans* and Lactobacilli species has been theorized

by Sims. Studies of these organisms and their growth habits suggested that in most situations Streptococci are initial colonizers over teeth. The acid condition created by *S.mutans* favors the presence of lactobacilli. In presence of sucrose, *S. mutans* form extracellular dextrans and levans which can be utilized later to produce lactic acid.^[8] Further, it is stated that *S.mutans* have a short generation time and multiply faster than lactobacilli. They are acidogenic, which results in accumulation of acid. As the pH falls, the generation time of *S.mutans* lengthens and becomes longer than lactobacilli. But when this occurs, Lactobacilli multiply more rapidly than Streptococci; the environment becomes more acidic and growth of *S.mutans* is inhibited. This shows that caries is initiated by *S.mutans*, whereas lactobacilli help further progression of the lesion inside the dentin.^[8]

Antimicrobial agents have been in the tradition that exert a direct bactericidal effect on caries producing bacteria. Many of these are the chlorhexidine mouthwashes,^[13] gels, sodium hypochlorite solutions, etc. Their regular and long-term use cannot be advocated due to the potential side effects. Thus, there arises a need to introduce an agent that is relatively safe and equally efficient in targeting the microbial etiology. BTEMR was thus studied to know the antibacterial action that can be exerted by it on salivary load of *S.mutans*.

Black tea is a fermented tea harvested from the sp. *Camellia sinensis*. Major difference between black tea and the other types of tea is that black tea contains highest amount of catechins which are the major components possessing the antibacterial properties.^[5]

These catechins make up almost 30 to 40% of the composition of dried black tea leaves^[14-17] and so brewing only 2 g of these leaves in 100 ml of water (i.e. 2% of BTEMR) was done in the present study. This yielded a concentration of around 6000 to 8000 µg/ml of the catechin compounds. These levels asserted to be well above the minimum inhibitory concentration of 250 to 1,000 µg/ml of the phenolic compounds of the black tea that is required to exert an antibacterial action.^[18-20]

Black tea components are the polyphenols which constitute the most interesting group amongst the components of black tea leaves. The main polyphenols in black tea are catechins (flavan-3-ols). The four main catechins are: epigallocatechin 3 gallate (EGCG) that constitutes about 59% of total catechins, epigallocatechin (EGC) about 19%, epicatechin 3 gallate (ECG) about 13.6% and epicatechin (EC) about 6.4%.

The constituents of black tea synergistically help in the inhibition of dental caries and can be summarized into following mechanisms:

1. Remineralisation of dental hard tissues
2. Inhibition of bacterial enzymes
3. Prevention of bacterial adherence
4. Direct bactericidal action.

Lemos J (2005) suggested that the suppression of F1Fo-ATPase and agmatine deiminase system(AgDS) by EGCg may lead not only to energy starvation but also to disruption of constant pH across the cell membrane, which in turn may trigger a series of physiological effects in the cell. As a result of suppression of AgDS and F1Fo-ATPase cytoplasmic acidification and impaired acid tolerance may inhibit the normal function of various acid intolerant enzymes. The optimum pH range of GTFs in *S.mutans* was reported to be 5.5 to 6.0. The malfunction of GTFs at the lower pH value may lead to reduced production of EPS and intracellular polysaccharides (IPS). The latter of which could have been metabolized when exogenous fermentable substrate was depleted in the oral cavity. Therefore, the malfunction of GTFs may disrupt both bacterial adherence to the tooth surface and biofilm integrity and may augment the starvation stress of *S.mutans* cells due to the reduced preservation of IPS.^[21] Cytoplasmic acidity, in concert with the inhibition of enolase by EGCg, may also inhibit the normal process of glycolysis, as described above. This in turn will diminish the ATP pool and further suppress the activity of the proton translocation (F1Fo-ATPase), aggravating cytoplasmic acidification.

The inhibition of LDH at both transcriptional and enzymatic levels may also increase the levels of NADH and decrease the redox potential of the cell, leading to the NAD⁺ /NADH imbalance and/or accumulation of glycolytic intermediates in the cell, which is toxic for *S.mutans*. The net result would be cytoplasmic acidification and disrupted glycolytic processes with diminished ATP pools, thereby triggering a series of cascaded biological effects at molecular levels, leading to compromised competence to environmental stress and impaired cellular functions, even cell death. Thus, Xu X et al summarized that EGCg represents a natural and alternative anticariogenic agent because (i) EGCg inhibits growth of both *S.mutans* planktonic and biofilm cultures, and (ii) EGCg inhibits various cariogenic virulence factors of *S.mutans* at the transcriptional and enzymatic levels, leading to reduced acidogenicity and compromised stress tolerance (especially acid tolerance).

On comparison of the salivary load of *S.mutans* from the culture plates of samples obtained before and after the administration of BTEMR, it was observed that there was a significant fall in the salivary *S.mutans* load after rinsing with BTEMR.

The present study thus reflects the antibacterial effect of BTEMR particularly on salivary *S.mutans* and that regular use of BTEMR can keep a check on the major cariogenic microorganisms like *S.mutans* by exerting its bactericidal action.

The black tea consumption orally in the form of a drink, although being safe for most of the people in moderate quantities, few adverse health effects like gastrointestinal upset and hepatotoxicity are possible.^[22]

CONCLUSION:

This study concluded that the effect of 2% BTEMR on salivary *S.mutans* load leads to significant reduction of the same irrespective of age or gender. This in turn implies that a regular use of BTEMR by the general population can prove to be an effective natural anticariogenic agent with no known implicated side effects.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Research Article

Marking the Micro Details in Potentially Malignant Disorders of Oral Cavity

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ABSTRACT:

Background- The malignancy of the oral cavity constitutes the most important group of malignancies in South and Southeast Asia. The risk increases with the use of tobacco products.

Materials & Methods- Micronuclei frequency scoring was used as a biomarker to identify different potentially malignant disorders.

Results- Mean micronuclei index was found higher using Hematoxylin and Eosin stain than Papanicolaou's stain and May Grunwald's stain.

Conclusion- We concluded that the micronuclei frequencies in oral exfoliated epithelial cells using three different stains- Hematoxylin and Eosin stain, Papanicolaou's stain, May Grunwald's stain may be useful in predicting the malignant potential of premalignant lesions.

KEYWORDS: papanicolaou's stain; may grunwald's stain; micronuclei

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INTRODUCTION:

Oral cancer is still a major global health concern even after major advance in understanding of carcinogenesis.^[1] Tobacco consumption is the most common preventable attribute for oral cancer disease. Tobacco-related disease kills approximately 6 million people in world; the figure is estimated to reach around 8 million by 2030. India being one of highest tobacco producer and consumer of tobacco-related products is at a verge of risk of being the country with high oral cancer patients. Tobacco paan masala, and tobacco with pan and betel quid are the most consumed form of SmokelessTobacco (SLT) in India.^[2] SLT is strongly associated with precursor lesions of oral cavity and esophageal cancers.^[3] Tobacco contains nicotine and carcinogens, including nitrosamines (*i.e.*, NNK and NNN). Nitrosamines cause epithelial cells' division

leading to focal growth and morphologic changes in the early stages in cell transformation.^[3] Accumulation of genetic alterations within oral epithelial cells/mucosa, induced by the genotoxins present in tobacco-related products often lead to oral potentially malignant disorders (PMDs).

These PMDs includes leukoplakia, erythroplakia, lichen planus and oral submucous fibrosis, are known to have an increased risk of Oral squamous cell carcinoma (OSCC). These lesions harbor genomic abnormalities in the form of micronuclei indicating enotoxicity in oral epithelium. The micronuclei index has become one of the standard cytogenetic biomarkers used in cancer biology. Many studies have indicated that there is an increased micronuclei index in PMD which are procurers of OSCC.^[4] Identifying the presence of

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micronuclei in oral epithelial cells in early stage can help clinicians in the initiation of preventive measures.

Exfoliative cytological study of oral cells is a non-aggressive technique and its application in the early diagnosis of PMDs is well-established. The assay is reliable and technically easy to perform. Early detection and early intervention of oral cancer can prolong life expectancy and reduce the years of life lost, indicating the importance of proactive screening and oral hygiene.

Numerous DNA specific stains available are used to study the micronuclei and chromosomal abnormalities within the cells. Many investigators investigated a variety of stains including DAPI, acridine orange (AO), Hoechst, and propidium iodide, Feulgen-Fast Green (FFG), May-Grunwald Giemsa (Giemsa) and Papanicolaou (Pap). Feulgen-Fast Green (FFG) which enable easy identification of Micronuclei, making it clearly stand among other stains. However, relatively lengthy procedures may result in the under-scoring of Micronuclei. Many comparative studies of micronuclei using stains were performed in the past. Casartelli et al. evaluated Hoechst, PI, and Giemsa, and found Hoechst was the more reliable for staining and identifying micronuclei. Giemsa, FFG, DAPI, and acridine orange stains' comparison show that Giemsa is more associated with falsification of micronuclei and FFG is better than other stains. The Papanicolaou (Pap) stain was the preferred method of detecting micronuclei in oral epithelial cells when compared to Giemsa stain. The fluorescent staining (acridine orange) was more sensitive for micronuclei detection than FFG in oral exfoliated cells in subjects of leukoplakia and squamous cell carcinoma. Studies are needed to determine whether some micronuclei and nuclei may lose DNA through karyolysis while maintaining the protein structure of chromatin and the nuclear envelope, so that they would still be detectable by stains that are not DNA-specific.

Looking at the present scenario, we decided to evaluate micronuclei index in exfoliated cells using Hematoxylin and Eosin stain (H&E), Papanicolaou's stain (PAP), May Grunwald's stain (MGG) among the OPMD's cell samples. The objective of study was: (a) to determine micronuclei index among OPMD's and OSCC, (b) to determine efficacy of H & E, PAP, MGG stains for staining and identifying micronuclei, (c) comparisons of H&E, PAP, MGG in OPMD's.

MATERIALS & METHODS:

The study was approved by the Research ethical committee, PCDS & RC and study was conducted at Department of Oral Pathology &

Microbiology. The subjects were pooled from the outpatient department based on criteria and divided into four groups as follows: (a) subjects with oral submucous fibrosis and later confirmed histopathologically as dysplastic lesions, (b) subjects with clinical leukoplakia and later confirmed histopathologically as dysplastic lesions, (c) subjects with clinical lichen planus and later confirmed histopathologically as dysplastic lesions(d) healthy subjects without history or habit of SLT chewing or any other form of tobacco consumption. Each group was comprised of 20 subjects. Written consent was obtained from subjects after explaining the purpose of study.

Collection of sample and processing:

Subjects were asked to rinse the oral cavity with saline before obtaining the cytology sample. Standard exfoliative cytology procedure was used to collect samples from site. The scrapes were taken with the help of a sterile metal spatula. The sample was transferred to a clean glass slide and fixed with alcohol. These slides were dried for 5-10 minutes and stained with H&E, PAP and MGG. All stained slides were examined at 1000X magnification using oil immersion objective under Binocular research Microscope (Olympus CH 20i, Olympus, India). Minimum 5 slides were prepared and screened for cell yield. Those with greater cell yield were selected for evaluation and counting of micronuclei. Minimum two slides for each stain per subject were counted. Micronuclei were counted as per Tolbert's criteria: (a) Rounded smooth perimeter suggestive of a membrane. (b) Less than a third the diameter of the associated nucleus, but large enough to discern shape and color, (c) Staining intensity like that of the nucleus, texture like that of nucleus, (d) Same focal plane as nucleus and absence of overlap with, or bridge to, the nucleus. Only those structures fulfilling the above-mentioned criteria were recorded as MNI (Figure 1). 720 slides were prepared, and 1000 cell/slide were counted for micronuclei. 7, 20,000 cells were counted in total. The gathered data was analyzed using unpaired t-test and chi-square test.

RESULTS:

The mean number of micronuclei were observed using H&E, PAP and MGG stain in subjects with OSMF. Mean micronuclei index was found high ($p<0.001$) using H & E stain (12.20) than PAP (8.20) and MGG (5.40)[Graph 1]. The mean number of micronuclei were observed using H&E, PAP and MGG stain in subject with leukoplakia. Mean micronuclei index was found high ($p<0.001$) using H & E stain

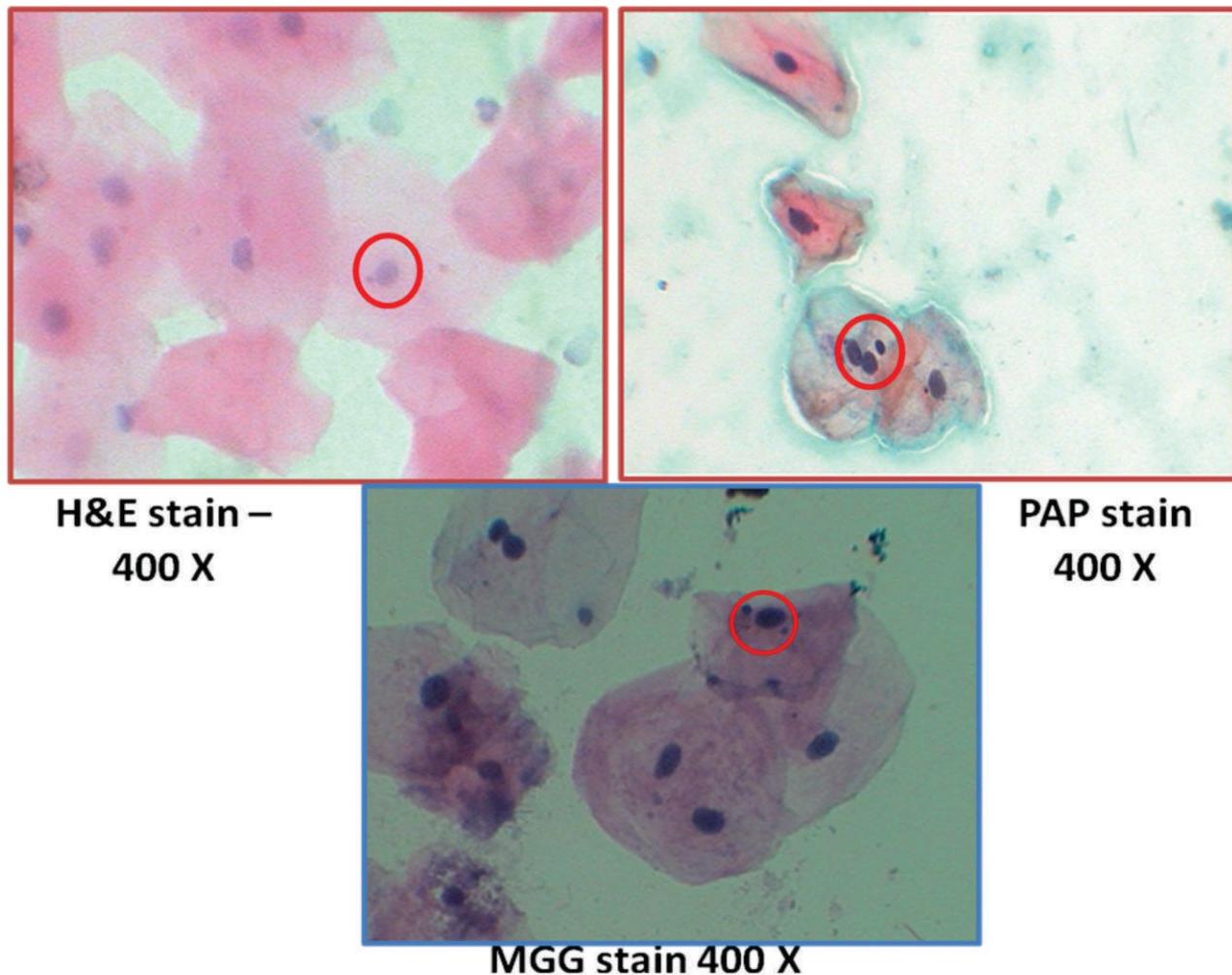
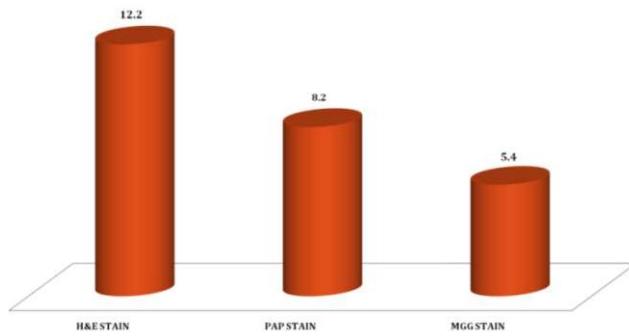


Figure 1: Photomicrographs showing micronuclei (circle) in oral exfoliated epithelial cells (a: H and E, b: Pap, c: MGG, $\times 1000$)

(18.00) than PAP (12.20) and MGG (5.80)[Graph 2]. The mean numbers of micronuclei were observed using H&E, PAP and MGG stain in subject with Lichen planus. Mean micronuclei index was found high ($p<0.001$) using H &E stain (8.0) than PAP (5) and MGG (2.2)[Graph 3].The mean number of micronuclei were observed using H&E, PAP and MGG stain in subjects with Control subjects. Mean micronuclei index was found high ($p<0.001$) using H &E stain (2.40) than PAP (1.60) andMGG (0.40)[Graph 4].The mean number of micronuclei were observed using H&E stain in subjects with OSMF, Leukoplakia, Lichen planus. Control Mean micronuclei index was found high ($p<0.001$) in Leukoplakia (12.20) when observed using H &E stain [Graph 5].

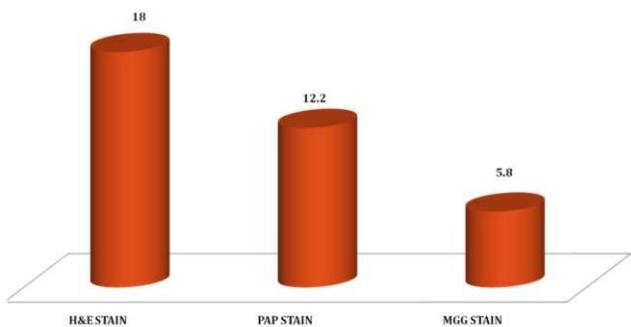
DISCUSSION:

Oral cancer is still a health burden in the world

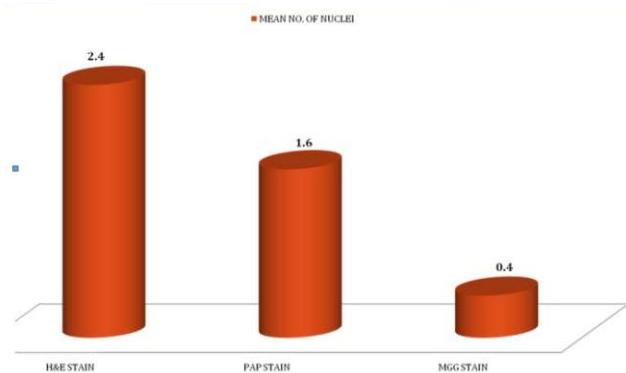


Graph 1: Mean no. of nuclei among three stains in OSMF patients.

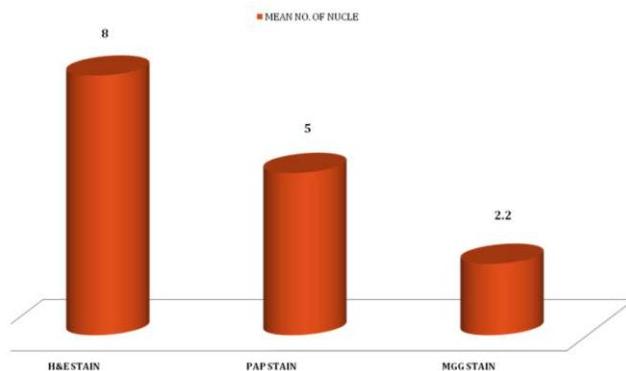
even in the 20th century. Even though there are many advances in detection and treatment, mortality and morbidity rates are high in many body cancers. Prevention is still the most effective way to reduce mortality and morbidity rate in the world. However, to detect and predict early premalignant changes in the tissue is still a dilemma among the pathologists, clinicians, and oncologists. The Etiology factor-based



Graph 2: Mean no. of nuclie among all three stains in LEUKOPLAKIA Patients.



Graph 4: Mean no. of nuclei among all three stains in CONTROL Patients.



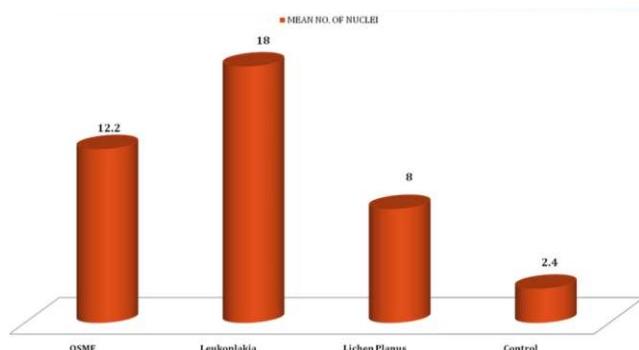
Graph 3: Mean no. of nuclei among all three stains in LICHEN PLANUS Patients.

approach is vague since carcinogenesis is a multifactorial and continuous evolving process.

Oral cancer is preceded by OPMD's and has variable rate of malignant transformation. Oral Leukoplakia is a common potentially malignant disorder affecting oral mucosa. The annual malignant transformation ranges of leukoplakia, Lichen planus and OSMF are 2% to 3%, 0.5%, 7.6 % respectively %^[5,6]

Currently, it's difficult to anticipate transformation of OPMD's that can be implemented to prevent such transformation. Cytology has been reliable, convenient, low cost, effective primary diagnostic testutilized all over the world. Cytological changes such as cytoplasmic granulation, cellular enlargement, vacuolization, pyknosis, binucleation, karyorrhexis, karyolytic, micronucleation, nuclear budding, nuclear enlargement is easily identified within exfoliated cell samples. Recently, the Micronuclei index has been suggested to be effective in cancer risk prediction, screening, diagnosis, and monitoring treatment progress^[7].

Cytology for micronuclei index has been used before to determine the genotoxicity due to SLT among PMD's. Various environmental, chemical, occupational



Graph 5: Mean no. of nuclei among all four lesion by H & E STAINING.

factors, lifestyle habits, can produce these changes among the oral mucosal cells.^[8] Among them tobacco-specific nitrosamines have been reported to be potent mutagenic agents which are thought to be responsible for the induction of chromosomal aberrations resulting in production of micronuclei.

Tobacco users have high micronuclei index^[9], which also plays role in development of OSCC.^[4] The effect of tobacco on buccal cells shows that there is chromosomal breakage and increase in DNA damage. Buccal micronucleus cytome assay has been suggested as a tool for biomonitoringDNA damage under HUMN project.^[10]

Our study has tried to resolve the issue of micronuclei counting among OPMD's, viz. OSMF, Leukoplakia, and Lichen planus. The present study found that leukoplakia has higher ($p<0.001$) micronuclei index as compared to the others. The results were significant. This is in accordance with what was observed by other researchers previously.^[11]

^[12] The tobacco-related DNA damage, which was more closely correlated with OSCC than OSMF and lichen planus, is due to tobacco smoking.^[13]

Areca nut, autoimmunity is usually associated with OSMF and lichen planus respectively. Our observations are like Mahimkar et al.^[14], indicating increased DNA damage in leukoplakia.

Various specific stains and non-specific DNA stains were used in evaluation of micronuclei in exfoliated buccal cells.^[11,15] Our results were in accordance with Grover et al and Katarkar et al that OPDM's show higher micronuclei counts than normal subjects as well as higher micronuclei count was seen with H &E stain. The micronuclei count in OPMD's was 5-fold as compared to control whereas Bloching et al.^[16] and Katarkar et al^[11] showed there was a 2.2 and 3.6-fold increase in the micronuclei frequency in leukoplakia as compared to normal subjects.

CONCLUSION:

The micronuclei count assay has potential to predict genotoxicity as well as malignant potential among the premalignancies since there are no other parameters available as of now. Our study concludes that Micronuclei cont. index can be helpful in predicting the malignant potential among the subjects even with the help of Non-specific H & E stain. H&E stain is routinely used, available and have easy practicability. However, one needs to train themselves in micronuclei counting procedure and acquire skills for accurate identification.

Financial Support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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Research Article

Effectiveness of Planned Teaching Programme on knowledge regarding prenatal factors leading to mental disorders among prenatal mothers in selected areas of Bhopal

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ABSTRACT:

Background- The mental illness seen in children is worrisome and has many factors leading to such conditions. The need for positive intervention is need of the hour.

Materials & Methods- Quasi-experimental one group pre-test post-test design was used for the study. The sample consisted of 60 prenatal mothers in selected rural areas of Bhopal by Non-Probability convenient sampling technique. A pre-test was conducted by using a structured knowledge questionnaire to evaluate the effectiveness of Planned Teaching Programme on knowledge regarding prenatal factors leading to mental disorders.

Results- In this study, on comparing the pre-test and post-test knowledge score it was found that all prenatal mothers scored better in the post-test. Majority of respondents scored average and 20.0% scored good. The post test mean was more than pre test score. **Conclusion-** We concluded that the structured teaching program is effective in increasing knowledge of the prenatal mothers regarding prenatal factors leading to mental disorders.

KEY WORDS: effectiveness; planned teaching programme ; knowledge; prenatal mothers.

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INTRODUCTION:

According to Health day news a new study suggests that pregnancy behaviour and certain childbirth complications may influence a child's risk of developing obsessive compulsive disorder. Certain C-sections, preterm and breech birth presentation, smoking while pregnancy and unusually large or small babies may be the factors responsible. While both genetic and environmental risk factors are thought

to be associated with increased risk for the mental health disorder, " While both genetic and environmental risk factors are thought to be associated with Obsessive Compulsive Disorder (OCD), this is the first time that a set of environmental risk factors is convincingly associated with the condition.^[1]

Attention deficit hyperactivity disorder (ADHD) has a worldwide prevalence of 2.5% with most symptoms appearing by the age of 6 years. General prenatal stress and stressful life events during

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pregnancy increase the risk of having a child who develops ADHD. Autism spectrum disorders affect 1-2% of the population and symptoms of poor sociability and communication skills repetitive behaviors, and circumscribed interests tend to become apparent by the age of 3 years. Inflammatory responses in the mother may affect fetal and postnatal brain development. Originally based on the knowledge that Autism Spectrum disorder (ASD) was associated with season of birth, exposure to viral infections has been shown to increase the risk of ASD in many but all studies.

It has been estimated that approximately one third of the children are suffering not from physical but from psychological illness. Identification and handling of the emotional, behavior and developmental disorder problem of the childhood and adolescence is very essential for any health care worker including nurses. The common behavior problem according to their nature of deviation in behavior includes Habits, Eating, Sleep, speech, Scholastic sexual, Antisocial Personality^[2]

WHO further states that the well-being of an individual is encompassed in the realization of their abilities, coping with normal stresses of life productive work and contribution to their community. Cultural differences, subjective assessments and competing professional theories all affect how "Mental Health is defined".^[3]

There are more than 200 classified forms of mental illness. Some of the more common disorders are Depression, Bipolar mood disorder, Dementia, Schizophrenia and anxiety disorder. Symptoms may include changes in mood, personality, personal habits and social withdrawal. As with cancer, diabetes and heart disease, mental illness is often physical as well as emotional and psychological. Mental illness is caused by reaction to environmental stress, genetic factors, biochemical imbalance, or a combination of these. With proper care and treatment may individuals learn to cope or recover from a mental illness or emotional disorder.^[4]

Primitive beliefs regarding disturbance have several views. Some thought that an individual with mental disorders had been dispossessed of his/her soul and that the only way wellness could be achieved was if the soul should return. Other believed that evil spirits or supernatural powers or magical power had entered the body. The cure of these individuals involved a ritualistic exorcism to purge the body of these unwanted forces.^[5]

In the medieval times, childhood was not regarded as a psychologically distinct period.

Childhoods were merely viewed as under developed adult at the fringes of society. In the 17th and 18th century philosophies, children were seen to have unique physiological, educational and physical need. In the 19th century a new approach to understand the childhood emerged. In the child guidance clinic, the common mental illnesses include mental retardation, conduct disorders, hyperkinetic syndrome enuresis etc.^[6]

Mental disorders have turned out to be major killer in Madhya Pradesh with 1,227 people suffering from such illnesses committing suicide in Madhya Pradesh in 2015, as per the National Crime Records Bureau (NCRB). As per the NCRB, Madhya Pradesh witnesses a 128% hike in such deaths in 2015 compared to 2014 when 538 such suicides were reported.

The present study was done to assess the knowledge regarding prenatal factors leading to mental disorders among prenatal mothers and to implement Planned Teaching Programme to prenatal mothers. The effectiveness of Planned Teaching Programme on knowledge regarding prenatal factors leading to mental disorder was determined.

MATERIALS & METHODS:

The prospective study was carried out in People's College of Nursing & Research Centre (PCN & RC), Bhopal. The study subjects were informed about the study and due consent was obtained from them. Ethical clearance certificate was obtained from the PCN&RC as per norms. The study involved 60 Prenatal mothers of selected rural areas of Bhopal. Interventional research approach was used in this study.

"One group, pretest post test research design" was selected which is a quasi-experimental design to evaluate the effectiveness of Planned Teaching Programme on knowledge regarding prenatal factors leading to mental disorders. Non-probability convenient sampling technique was used for the study. The duration of data collection was for 1 and half month. The inclusion criteria were that the prenatal mothers should understand Hindi language. Prenatal mothers who were not willing to participate were excluded from the study. In this study dependent variable was the knowledge of prenatal mothers regarding prenatal factors leading to mental disorders whereas independent variable are Planned Teaching Programme regarding prenatal factors leading to

mental disorders.

A structured questionnaire was designed for the study. It was divided into 2 parts. First part consisted of demographic questionnaire that includes age, education, type of family, religion, and source of information regarding mental health and occupation.

The second part consisted of 30 Multiple choice Questions to evaluate the knowledge regarding prenatal factors leading to mental disorders. The validity of the questionnaire was confirmed.

The sample questionnaire is depicted in Figure 1. Broadly the questions were aimed to ascertain the knowledge regarding mental health & mental illness, prenatal factors leading mental disorders and prevention of mental illness.

RESULTS:

The demographic data revealed that majority of prenatal mothers were between 24 to 29 years [Table 1]. Majority of Prenatal mothers approximately 46% were primary educated [Table 2]. 58% belonged to joint family [Table 3] and 76% were Hindu [Table 4].

Very few prenatal mothers have access to either television or newspaper. Around 43% have no access to any kind of media [Table 5]. Most (58.4%) worked as laborer followed by housewife [Table 6]. The study finding reveals that the mean post-test knowledge score (15.8) is higher than the mean pre-test knowledge score (8.8) [Table 7].

Table 1: Frequency and percentage distribution of prenatal mothers according to Age in years.

Age in years	Number	Percentage
18-23 yrs	21	35.0
24-29 yrs	29	48.3
30-35 yrs	10	16.7
More than 35 yrs	00	00
Total	60	100.0

DISCUSSION:

A child is the purest form of a human being as he is not yet molded by the harsh realities of life. As each child looks at the world through innocent eyes all he can see, the way of life and the way he think life should be. We owe our children, the most vulnerable citizens in our society, they are like flowers and they fill our life with joy and fragrance.

Table 2: Frequency and percentage distribution of prenatal mothers according to Education.

Education	Number	Percent
Primary	28	46.7
Secondary	18	30.0
Higher Education	13	21.7
Graduate	1	1.7
Total	60	100.0

Table 3: Frequency and percentage distribution of prenatal mothers according to type of family.

Type of family	Number	Percent
Joint family	35	58.3
Nuclear family	19	31.7
Extended family	6	10.0
Single parent family	00	00
Total	60	100.0

Table 4: Frequency and percentage distribution of prenatal mothers according to Religion.

Religion	Number	Percent
Hindu	46	76.7
Muslim	14	23.3
Christian	00	00
Others	00	00
Total	60	100.0

Table 5: Frequency and percentage distribution of prenatal mothers according to source of information.

Source of information	Number	Percent
Television	15	25.0
Health Professional	13	21.7
Newspaper	6	10.0
None of the above	26	43.3
Total	60	100.0

Table 6: Frequency and percentage distribution of prenatal mothers according occupation.

Occupation	Number	Percent
House wife	23	38.3
Private Job	2	3.3
Government job	0	0
Labourer	35	58.4
Total	60	100.0

Proper education should be given to the mothers to make them vigilant towards the prenatal factors leading to mental disorders of child. Nurses process a major obligation in the education, motivation, and also in the guidance of the mentally ill children by that, she can help them to stay away from mental illness. The present study was aimed to evaluate effectiveness of teaching program on knowledge regarding prenatal factors leading to mental disorders among the prenatal mothers of selected rural areas of Bhopal.

A Structured knowledge questionnaire was used to collect the data. The participants were grouped as pre-test and post-test to evaluate the knowledge of 60 prenatal mothers. After the administration of pretest Planned Teaching Program was administered, post-test knowledge was determined after 7 days using same structured knowledge questionnaire to find out the effectiveness. We found that pre-test mothers in selected areas of Bhopal were not having knowledge regarding prenatal factors leading to mental disorders. We also determined that Planned Teaching Programme is an effective intervention to enhance the knowledge of prenatal mothers.

CONCLUSION:

The study was conducted with the objective to evaluate the effectiveness of Planned Teaching Programme knowledge regarding prenatal factors leading to mental disorders among prenatal mothers. We concluded that with the intervention of Planned teaching program amongst prenatal mothers, their knowledge increased which could have positive impact on reducing mental illness.

Financial Support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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Research Article

An Exploratory Study to Assess the Knowledge & Attitude of Parents Regarding Play Needs of Children (under 5 years of age) in Selected Hospital of Durg (C.G.)

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ABSTRACT:

Background-The human life has a distinct sphere—the world of play makes childhood more meaningful, happy & motivate the child to learn, develop & mature. Parents, teachers, nurses, psychologists are becoming increasingly aware of the importance of play and its influence upon bringing of children. The study was designed to assess the knowledge and attitude of parents regarding play needs of children.

Materials & Methods- 100 couples were selected using purposive sampling technique. A structured questionnaire was prepared for assessing the knowledge & attitude of parents regarding play needs of children (under 5 years of age).

Results- 20% of them had moderately adequate knowledge whereas 22% had moderately adequate attitude with. Knowledge & attitude of parents correlated. There is no significant association between socio demographic variables and knowledge except gender, religion, and mass media exposure, type of family, and monthly income and number of children as demographic variables. There is no significant association between socio demographic variables and attitude except gender, religion, qualification, type of family, and number of children as demographic variables.

Conclusion-This study was conducted in Govt. Hospital of Durg (Chattisgarh) with the parents having children under 5 years of age. The findings of the study recommended the further interventional approaches regarding play needs of children. Parents need to be educated about meaning and importance of play for child. It creates awareness play know, attitudes, play needs, under five.

KEYWORDS: pregnancy; brittle bone disease; osteogenesis imperfecta; materna outcome; fetal outcome.

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INTRODUCTION:

Play is a dynamic, active, constructive behavior and essential and integral part of all children's healthy growth, development, and learning across all domains, ages and cultures. Play is a dynamic process that develops and changes as it becomes increasingly more varied and complex. It is considered a key facilitator for learning and development across all domains and reflects the social and

cultural contexts in which children live. Playing is learned by so many factors like proper health (mental & physical), motor development, sex, surrounding environment, socioeconomic status and play equipment/items. Play is that accepted part of a child's life today that few people refuse to consider the role it plays in the child development. Play is basically categorized into two parts; namely: *Active play* in which the child is actively involved in games and the other

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Table 1: Demographic details.

S. No.	Demographic variables	No. of parents (n)	%
[1] Age			
1.1 31 -35 yrs	63	63.0%	
1.2 36 -40 yrs	24	24.0%	
1.3 41 -45 yrs	9	9.0%	
1.4 46 -50 yrs	4	4.0%	
[2] Religion			
2.1 Hindu	65	65.0%	
2.2 Muslim	16	16.0%	
2.3 Christian	11	11.0%	
2.4 Sikh	8	8.0%	
[3] Sex			
3.1 Male	40	40.0%	
3.2 Female	60	60.0%	
[4] Qualification			
4.1 Illiterate	27	27.0%	
4.2 Primary/middle education	41	41.0%	
4.3 High school/ higher secondary education	19	19.0%	
4.4 Graduate//post graduate education	13	13.0%	
[5] Mass media			
5.1 Radio	17	17.0%	
5.2 TV	42	42.0%	
5.3 Magazine	14	14.0%	
5.4 Newspaper	27	27.0%	
[6] Type of family			
6.1 Nuclear family	61	61.0%	
6.2 Joint family	39	39.0%	
[7] Monthly income			
7.1 < Rs.3000	47	47.0%	
7.2 Rs.3000 -5000	18	18.0%	
7.3 Rs.5000 -7000	21	21.0%	
7.4 Rs.7000 -10000	14	14.0%	
[8] No. of children			
8.1 One	21	21.0%	
8.2 Two	35	35.0%	
8.3 Three	26	26.0%	
8.4 >Three	18	18.0%	

being **Passive play** in which the child enjoys other's activities for e.g.: watching people & animal on T.V. Our study assessed the association between play need of children and attitudes of parents towards play of children under the age 5.

MATERIALS & METHODS:

The study was carried out in Government hospital in Durg (Chhattisgarh). Ethical permission was duly taken. The participants were informed about

the study. Non experimental research design was used to assess the knowledge & attitude of parents regarding play needs of children (under 5 years of age). 100 couples (parents) were selected using purposive sampling technique. All the couples willing for the study and falling within 31 to 50 years were included in the study. Validity and reliability of tool were tested and a self-structured questionnaire was prepared. 31 - 50 years age group of parents were included in study. After collection of data, informational booklet was

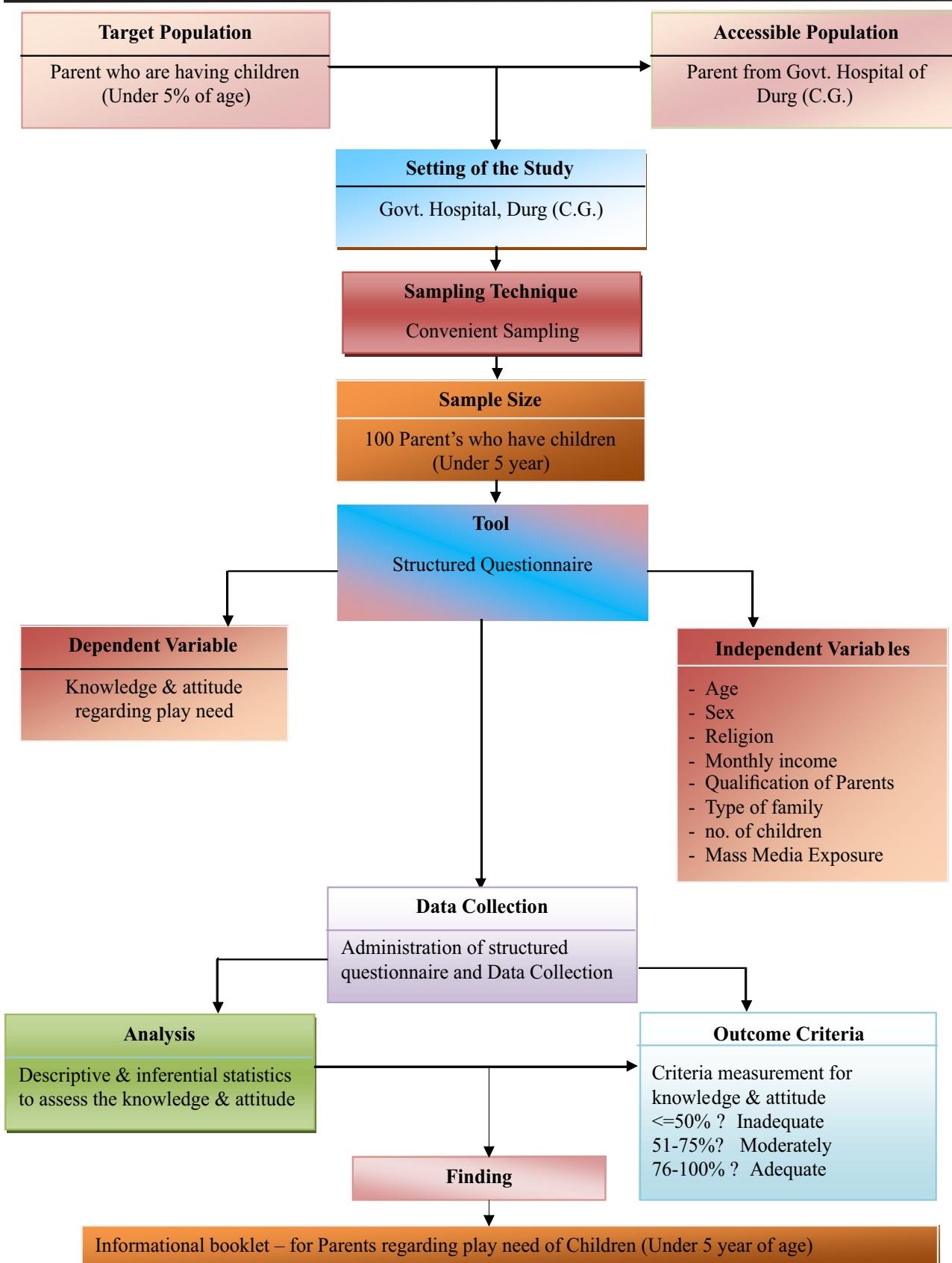


Figure 5: Schematic representation of the research design.

Table 2: Parents level of knowledge score on play needs of children

Level of knowledge	Criteria for measurement	No. of parents	%
Inadequate knowledge	0 - 11.5 = <= 50%	80	80.0%
Moderately adequate knowledge	11.6 - 16.5 = 51 - 75%	20	20%
Adequate knowledge	16.6 - 22.0 = 76-100%	0	0.0%
Total		100	100%

Table 3: Parents level of attitude score on play needs of children

Level of Attitude	Criteria for measurement	No. of parents	%
Inadequate knowledge	0 - 1 = <= 50%	78	78.0%
Moderately adequate knowledge	11 - 15 = 51 - 75%	22	22%
Adequate knowledge	16 - 20 = 76-100%	0	0.0%
Total		100	100%

distributed. Booklet explained the importance of play in child, advantages & types of play, playing activities according to age and suitability of toys.

Firstly, demographic details such as age, religion, qualification, mass media exposure, type of family (nuclear or joint), monthly family income and number of children were assessed. This was followed by assessing the level of knowledge parents had regarding the play needs of children under the age of 5. There was a scale to assess the same. If the parents' knowledge were less than 11.5, the parents had inadequate knowledge. If the score was between 11.6 to 16.5, the parents had intermediate knowledge and if the scores were above 16.6, the parents had good knowledge. Similarly, the attitude of parents towards play needs were assessed. The criteria for its measurements were as follows- scores less than 11 were grouped as inadequate knowledge, between 11 to 15 were grouped as moderate knowledge and finally 16 and above were grouped as good knowledge. Figure 1 depicts the strategy for the study.

RESULTS:

63% of parents were in the age group of 31 to 35 while only 4% were within 46 to 50 years. 65% were Hindu being maximum in numbers. 73% have had some kind of formal education. Regarding mass media exposure, all couples had access to atleast some form of media. Around 61% were a nuclear family. Most of the couples (47%) had a monthly income less than Rs 3000 per month. 35 % had 2 children followed by couples having 3 children [Table 1].

The level of knowledge of parents on play needs of children (under 5 year of age) showed that 80% of the parents were having inadequate knowledge

and 20% of them had moderately adequate knowledge and none of them had adequate knowledge [Table 2].

The level of attitude of parents on play needs of children (under 5 year of age) showed that 78% of the parents were having inadequate attitude and 22% of them had moderately adequate attitude and none of them had adequate attitude [Table 3].

DISCUSSION:

The data was collected, gathered, analyzed and interpreted in terms of objectives of the study. The mean, standard deviations, mean percentage, mean difference percentage of knowledge & attitude of parents regarding play needs of children (under 5 years of age) was analyzed by using descriptive and inferential statistics.

Finding of the study revealed that only 24 % gave correct responses. 73 % parents had no knowledge about rights of child. 72% parents did not agree that play should be fundamental part of every school curriculum but 28 % parents agreed. 69% parents did not know that play is helpful for self-confidence where 31% were aware. 68% parents did not agree that play is a source of learning and 32% parents agreed. 65% did not believe that lack of parent's knowledge affects child's play. 68% parents did not believe that play helps to improve communication skill and 32% parents.

Verma M et al conducted a pre-experimental study to identify the knowledge & practice of nursing personnel regarding therapeutic play for hospitalized children in pediatric unit on the basis of planned teaching program. The finding of knowledge showed that, the mean pretest knowledge score was 15.75 with median 16 and standard deviation 2.79 against the

maximum score of 30. Range of obtained Score in pretest was between 10-22, indicating there was knowledge deficits regarding therapeutic play for hospitalized children. The mean post-test knowledge score was 22.63 with median 22 and standard deviation 2.40 against the maximum score of 30. The mean difference between pretest and post-test knowledge score was 6.9. This obtained mean difference was found to be statistically significant. This indicated that the planned teaching programmed was effective to enhance knowledge of nursing personnel regarding therapeutic play. Our results were similar to the study.

Sreedhar Teji studied mother's knowledge in anganwadi regarding play amongst children. The researcher concluded that the lowest mean of pretest score was found in the area of parental responsibility followed by developmental value (30%) developmental characteristics of play (33.33%), play item & activities (35%), meaning and importance of play (38.34%). The mean post-test knowledge score (42) was found to be significantly higher than the mean pretest score (16.4) with 't' (39)> 4.75 significant at 0.01 level. This suggested that the health education program was effective in increasing the knowledge of mothers regarding play in children. The mean percentage gain between pre-test & post-test knowledge score in all the content area indicated gain in knowledge in all the areas. The maximum mean gain score had been in the area of parental responsibility (51.83%) and minimum mean score was in the area of development characteristics of play. Knowledge score also revealed that maximum modified knowledge gain score was in the area of play activity and item (0.74%) followed by parental responsibility (0.69%) meaning & importance of play (0.68%) developmental value of play (0.64%) and developmental characteristic of play (0.57%). This was in accordance to our study.

Half the parents thought that play is very essential for child. 46% parents did not believe that fulfillment of play need is. Only 27% parents agreed that economic factor in family affect the play activities of child. 48% parents did not think feel the need of more knowledge regarding play need of a child. 43% of parents think cultural impact play role on playing. 33% think responsible parent's role during child's play activities is the need of the hour. Surprisingly, 66% parents do not think play is helpful for improving moral standard of a child. 39% parent think parent's encouragement is necessary in play activities. 33% parents believe that play is helpful for overall personality development. 57% parents did not think that parent's qualification is helpful for maintaining play needs of a child. 43% parents consented that play

creates positive thoughts in child. 59% parents were of the opinion that play helps in social, cultural & spiritual development of child. Lastly, 33% of parents think that play helps in improving communication skills.

The present study revealed that there was significant association 2=14.66 ($p=0.001$) between age & attitude of parents, in mass media 2=13.1($p=0.05$) and in monthly income of parents 2=8.66 ($p=0.03$).

CONCLUSION:

The present study showed that the maximum parents were having inadequate knowledge regarding play needs of children (under 5 years of age). Only age and education qualification variables are significantly associated with their knowledge score. Awareness programs are needed to help understand the role of play in a child's life.

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Conflicts of interest

There are no conflicts of interest.

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Research Article

A Study to Assess the Knowledge and Attitude Regarding Myths About Covid-19 Vaccination in Selected Rural Area Ratua at Bhopal (M.P.)

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ABSTRACT:

Background- COVID-19 is the Pandemic disease caused by the SARS-COV-2 virus. There are many myths regarding COVID-19 vaccine spread by community people at rural area. The objective of this study was to assess the knowledge about myths of COVID-19, Bhopal Ratua (M.P). The World Health Organization (WHO) is working closely with global experts, governments, and partners to rapidly expand scientific knowledge on this new virus, to track the spread and virulence of the virus, and to provide advice to countries and individuals on measures to protect health and prevent the spread. The urban people have poor attitude regarding myths about Covid- 19 vaccination.

Materials & Methods- A Study was conducted to assess the knowledge and attitude regarding Covid Vaccination among rural community people. 30 rural community people from a selected rural community were included in the study. A questionnaire consisting of 40 questions was used.

Results- Almost all had some pre-test knowledge regarding the myths surrounding Covid-19. Whereas the knowledge was increased post-test.

Conclusion- We concluded that such awareness programs may have a positive impact on increasing the knowledge of rural people.

After that the vaccine needs to go through a review by the National Regulatory Authority, who will decide if the vaccine is safe and effective.

KEYWORDS: COVID 19; vaccines; myths; respiratory syndrome; corona.

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INTRODUCTION:

The coronavirus disease 2019 (COVID-19) emerged in Wuhan, China at the end of 2019. Since then, it has spread to 200 countries and has been declared a global pandemic by the World Health Organization (WHO). To date, there are more than 2.3 million positive COVID-19 cases recorded with at least 150,000 deaths globally. The first cases of COVID-19 in India were reported in the towns of

Thrissur, Alappuzha, and Kasargod, all in the state of Kerala, among three Indian medical students who had returned from Wuhan. Lockdowns were announced in Kerala on 23 March and in the rest of the country on 25 March. By mid-May 2020, five cities accounted for around half of all reported cases in the country: Mumbai, Delhi, Ahmedabad, Chennai, and Thane^[1]. On 10 June, India's recoveries exceeded active cases

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for the first time Infection rates started to drop in September, along with the number of new and active cases. Daily cases peaked in mid-September with over 90,000 cases reported per day, dropping to below 15,000 in January 2021.

India began its vaccination program on 16 January 2021. As of 25 May 2021, the country had administered over 200 million vaccine doses. Scientists and researchers throughout the world have been working relentlessly to find a way to get rid of the lethal disease. About 2-3 million deaths per year have been avoided by vaccination^[2]. In pandemics such as the 1957, 1968, 1976, and 1977 outbreaks and the H5N1 outbreak (1997-1998), and the 2009 H1N1 outbreak, many vaccines were developed^[3&4]. With the approval of vaccines for COVID-19, it was expected that the pandemic can be controlled. The discovery of vaccination is considered as one of the great human achievements when it comes to maintaining public health.^[5-8] vaccination is the most effective way of controlling infectious diseases, yet success is challenged by individuals and groups who choose to delay or refuse vaccines.^[9] Acceptance of a vaccine or hesitancy has great public health implications as these partly determine the extent to which people are exposed to infections that could have otherwise been prevented.^[10] Hesitancy to be vaccinated can be driven by several reasons such as negative medical family experiences that are related to vaccinations of the parents, concerns about the safety of the vaccines, and religious or ethical reasons.^[11] Research shows that vaccine uptake can be influenced by several factors.^[12] Smith et al. found an association between vaccine uptake and not perceiving vaccines to cause adverse effects, general positive attitude towards vaccination, positive vaccine recommendations, perceiving fewer practical difficulties of vaccination, perceived susceptibility to illness, knowledge about the vaccine, social influences, trust in the health-care profession, and having increased information about the vaccine. Safety concerns, perceived low severity of illness, lack of awareness, and belief in alternative medicine are the common reasons for hesitancy towards vaccination,^[17] whereas healthcare professionals' advice, advice from friends and family members, self-protection, belief that vaccination is mandatory, and being responsible have been reported to be the factors affecting the general population's support for vaccination.^[13] Smith et al. found factors such as having previously been vaccinated, perceiving the vaccine to be effective, and perception of susceptibility to the disease to be strongly positively associated with the uptake of childhood

influenza vaccine in England while the factors strongly negatively associated with uptake included perceiving the vaccine to be unsafe, to cause short-term side effects or long-term health problems and believing that yearly vaccination may overload the immune system. A study of the perceived risks of vaccines in European populations revealed that the primary area of concern was vaccine safety, followed by perceptions of a low likelihood of contracting vaccine-preventable diseases, perceived low severity of vaccine-preventable diseases, beliefs that vaccines do not work, and overall lack of information. It is believed that children's uptake of the vaccine is influenced by the socio-economic characteristics of their parents^[15]. We have conducted this study to assess the knowledge and attitude regarding myths about Covid-19 vaccination in selected rural areas at Bhopal.

MATERIALS & METHODS:

A Descriptive Co-relational Study was conducted to assess the knowledge and attitude regarding Covid Vaccination among rural community people of Bhopal, Madhya Pradesh. A review of the literature and discussion with experts helped the investigator to develop an appropriate tool for data collection and design the methodology for the study. Data was collected using a knowledge questionnaire, which consisted of 30 items and 20 attitude statements. The tool was prepared by the investigator and validated by experts. Reliability was established by the split-half method using Karl Pearson's correlation formula. The reliability obtained was 0.85, which proved that the tool was reliable. A pilot study was conducted on 6 rural community people. This gave the basis for the investigator to conduct the main study. The main study was conducted on 30 rural community people from a selected rural community from 1st June to 20th June 2021. The obtained data were analyzed considering the objectives and hypothesis using descriptive and inferential statistics. The purpose of present study was to assess the knowledge and attitude of rural people regarding myths about Covid - 19 Vaccination, in a selected rural area of Bhopal, Madhya Pradesh. We included participants that were present during the study period and excluded those who were not willing. The questionnaire consisted of 2 sections A & B).

Section A: Consisted of 10 demographic variables like – age, sex, religion, qualification of male, qualification of female, occupation of male, occupation of female,

Table 1: Demographic distribution of the participants.

Variables	Frequency	Percentage
Age (Yrs)		
15-17	1	3.33333333
18-25	6	20
26-45	14	46.6666667
46 & above	9	30
Gender		
Male	16	53.3333333
Female	14	46.6666667
Transgender	0	0
Religion		
Hindu	13	43.3333333
Muslim	10	33.3333333
Christian	1	3.33333333
Other	6	20
Qualification of male		
Primary	7	46.6666667
Higher sec.	1	6.66666667
UG and PG	1	6.66666667
Others	7	46.6666667
Qualification of female		
Primary	3	20
Higher sec.	2	13.3333333
UG and PG	4	26.6666667
Others	5	33.3333333
Occupation of male		
Govt. job	0	0
Private job	4	26.6666667
Semi private job	3	20
farmer	9	60
Occupation of female		
Housewife	10	66.6666667
Govt job	1	6.66666667
Private job	2	13.3333333
Semi private job	1	6.66666667
Monthly income (Rs)		
<5000/-	6	20
5001 -10000/-	15	50
10001 -20000/-	9	30
>20000/-	0	0
Area of residence		
Urban	0	0
Rural	30	100
Semi-urban	0	0
Type of family		
Nuclear	19	63.3333333
joint	9	30
extended	2	6.66666667

area of residency, type of family.

Section B: Consisted of structured knowledge and attitude questionnaire containing 30 questions. Each right answer carries 1 mark and wrong answer carries 0 marks. Maximum score that can be achieved is 30. Knowledge level was then classified according to percentage of score. knowledge scores were determined.

Association between pretest knowledge and demographic variables were calculated by chi square test. Mean & standard deviation of pretest and post-test knowledge scores were determined.

RESULTS:

The prospective study was carried out in a rural district of Madhya Pradesh. After obtaining the ethical clearance, the participants were identified. They were explained about the study. 30 individuals formed the study group. Most of the participants were between 26 to 45 years and there were almost equal number of males and females. None of the participants were from government sector [Table 1].

This was followed by checking the pre-test knowledge. The results showed that most of the participants had some basic knowledge regarding the myths of Covid-19 vaccination [Table 2]. The post test knowledge results were very encouraging. It revealed that almost 83% participants improved their knowledge [Table 3].

Table 2: Assessment of pretest knowledge of the participants

Criteria	Frequency	Percentage	Mean	SD
Poor	12	40		
Average	17	56.66667	13	4.26
Good	0	0		

Table 3: Assessment of post-test knowledge of the participants

Criteria	Frequency	Percentage	Mean	SD
Poor	02	6.66666%		
Average	03	10%	27.6	1.81
Good	25	83.333%		

Table 4: Effectiveness of knowledge.

Test	Mean	SD	t-test
Pretest	13	4.26	1.651
Posttest	27.6	1.81	

Table 5: Association of knowledge of participants with demographic variables.

Variables	Poor	Average	Good	Total	DF	chi-value	P-value	Inference
Age (Yrs)								
15-17	0	0	1	1				
18-25	4	2	0	6	6	60	0.066	S
26-45	6	8	0	14				
46 & above	2	7	0	9				
Gender								
Male	10	5	1	16				
Female	2	12	0	14	4	11.63	0.033	NS
Transgender	0	0	0	0				
Religion								
Hindu	6	7	0	13				
Muslim	6	3	1	10	6	11.63	0.033	NS
Christian	0	1	0	1				
Other	0	6	0	6				
Qualification of male								
Primary	5	1	1	7				
Higher sec.	1	0	0	1				
UG and PG	1	0	0	1	6	13.52	0.056	S
Others	1	6	0	7				
Qualification of female								
Primary	2	1	0	3				
Higher sec.	1	0	1	2				
UG and PG	4	0	0	4	6	11.63	0.077	S
Others	0	5	0	5				
Occupation of male								
Govt job	0	0	0	0				
Private job	2	1	1	4	6	8.63	0.012	NS
Semi private job	2	1	0	3				
farmer	2	7	0	9				
Occupation of female								
Housewife	4	5	1	10				
Govt job	1	0	0	1	6	7.63	0.22	NS
Private job	1	1	0	2				
Semi private job	1	0	0	1				
Monthly income(Rs)	0							
<5000/-	3	2	1	6				
5001 -10000/-	6	9	0	15	6	6.55	0.033	NS
10001 -20000/-	3	6	0	9				
>20000/-	0	0	0	0				
Area of residence								
Urban	0	0	0	0				
Rural	12	17	1	30	4	3.55	0.55	NS
Semi-urban	0	0	0	0				
Type of family								
Nuclear	9	9	1	19	4	4.56	0.066	S
joint	3	6	0	9				
extended	0	2	0	2				

S=Significant; NS= Not significant

Table 9: Association of pretest attitude of the person with selected demographic variables.

Variables	Strongly agree	Agree	Disagree	Strongly disagree	Total	DF	Chi-value	p-value	Inference
Age (Yrs)	0								
15-17	0	1	0	0	1				
18-25	0	1	5	0	6	9	30	0.056	S
26-45	0	3	11	0	14				
46 & above	0	1	8	0	9				
Gender									
Male	0	3	13	0	16				
Female	0	3	11	0	14	6	12.36	0.022	NS
Transgender	0	0	0	0	0				
Religion									
Hindu	0	1	12	0	13				
Muslim	0	2	8	0	10	9	8.23	0.032	NS
Christian	0	1	0	0	1				
Other	0	2	4	0	6				
Qualification of male									
Primary	0	1	6	0	7				
Higher sec.	0	1	0	0	1				
UG and PG	0	1	0	0	1	9	11.52	0.075	S
Others	0	4	3	0	7				
Qualification of female									
Primary	0	1	2	0	3				
Higher sec.	0	1	1	0	2	9	7.52	0.063	S
UG and PG	0	2	2	0	4				
Others	0	2	3	0	5				
Occupation of male									
Govt. job	0	0	0	0	0				
Private job	0	1	3	0	4	9	8.63	0.012	NS
Semi private job	0	1	2	0	3				
farmer	0	4	5	0	9				
Occupation of female									
Housewife	0	4	6	0	10				
Govt. job	0	0	1	0	1				
Private job	0	1	1	0	2	9	6.69	0.11	NS
Semi private job	0	1	0	0	1				
Monthly income(Rs)									
<5000/-	0	2	4	0	6				
5001 -10000/-	0	3	12	0	15	9	5.63	0.022	NS
10001 -20000/-	0	1	8	0	9				
>20000/-	0	0	0	0	0				
Area of residence									
Urban	0	0		0	0				
Rural	0	6		0	30	6	2.52	0.45	NS
Semi-urban	0	0		0	0				
Type of family									
Nuclear	0	3	12	0	19				
joint	0	3	6	0	9	6	3.33	0.062	S
extended	0	0	0	0	2				

S=Significant; NS= Not significant

The results also depicted that the effectiveness of knowledge of the person regarding myths surrounding Covid-19 is statistically tested by applying student t-test at the level of significance of 0.05. In our study the calculated value 1.651 is less than table value <0.05 and so the hypothesis is accepted [Table 4]. The association of knowledge of the participants regarding myth about Covid-19 with selected demographic variables was statistically tested. The variables such as age, qualification of male and female and family type was found significant. The other variables were found non-significant [Table 5]. Assessment of pre-test attitude & post-test attitude of person regarding myth Covid-19 showed that 24 participants disagreed & 18 participants agreed respectively. Mean and SD also justified that attitude. [Table 6 & Table 7]. The effectiveness of attitude of the person regarding myth of Covid-19 was statistically tested by applying student t-test at the level of significance of 0.05. we found that the value 1.227 was less than table value <0.05 so the hypothesis is accepted [Table 8]. The association of knowledge of the person regarding myth about Covid-19 with demographic variables was statistically tested by applying chi-square test. The variables age, qualification of male and female and family type were found significant. Other variables were non-significant [Table 9].

Table 6: Assessment of pretest attitude of the participants

Criteria	Frequency	%	Mean	SD
Strongly agree	0	0		
Agree	6	20	13.75	2.85
Disagree	24	80		
Strongly disagree	0	0		

Table 7: Assessment of posttest attitude of the participants.

Criteria	Frequency	%	Mean	SD
Strongly agree	11	36.66667		
Agree	18	60	21.5	3.014
Disagree	1	3.333333		
Strongly disagree	0	0		

Table 8: Effectiveness of attitude of the participants

Test	Mean	SD	t-value
Pretest	13.73	2.85	1.227
Posttest	21.5	3.014	

DISCUSSION:

The study was intended to evaluate the effectiveness of information knowledge attitude regarding myths about COVID-19 vaccination in Ratua area at Bhopal (M.P.).

To achieve the objectives, pre-test & post-test was conducted.

Self-structure questionnaire was used to collect data on knowledge and attitude regarding myths about COVID-19 vaccination. There have been various studies and our results were in accordance to them.^[9]

CONCLUSION:

There was no significant association between knowledge score and attitude score with selected demographic variable like age, gender, religion, education of male, education of female, occupation of male, occupation of female, monthly income, type of family. Methods to ascertain the general beliefs and attitudes about Covid-19 vaccine should be standardized and health care officers and administrations should validate the questionnaires regarding the myths surrounding Covid-19. This shall enable overall acceptance of vaccines for life threatening diseases such as Covid-19.

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Conflicts of interest

There are no conflicts of interest.

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Review Article

Unsupervised Medical Abortions: A Cause for Concern of Safety

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ABSTRACT:

Medical abortions have been a national as well as social concern for the society, especially in India. There have been certain laws, acts and amendments that have been designed to promote safe abortions. Despite, the rules in place, there is still need to have more awareness as the women still face difficulties in accessing abortion services. The reasons may include legal hurdles, stigma, financial concerns and many more. Through this article, we would like to emphasize the role of government, medical professionals, health care providers to come together and form more dependable guidelines that may help women to access safer abortions.

KEY WORDS: abortions; MTP Act; contraception, abortion pills

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INTRODUCTION:

Women have the right to access safe abortion services. The right to safe abortion is grounded in principles of bodily autonomy, reproductive rights, and women's health. Access to safe and legal abortion services is essential to protect the health, well-being, and human rights of women.

The World Health Organization (WHO) recognizes that safe abortion services should be available to every woman who needs them. Access to safe abortion means that women can terminate a pregnancy in a legal and medically safe environment, with trained healthcare providers, using evidence-based methods and practices. Safe abortion services help prevent maternal morbidity and mortality associated with unsafe abortion procedures^[1].

International human rights frameworks, such as the Convention on the Elimination of All Forms of Discrimination Against Women (CEDAW) and the International Covenant on Economic, Social, and Cultural Rights (ICESCR), also emphasize the importance of ensuring access to safe abortion services

as part of women's reproductive rights and healthcare.

Governments and healthcare systems need to provide access to safe abortion services, following national laws and policies, to ensure that women can make informed decisions about their reproductive health and have access to the necessary care and support. This includes access to counselling, information, contraception, and post-abortion care.

Promoting access to safe abortion services is not only a matter of human rights but also contributes to public health and social well-being. It reduces the incidence of unsafe abortions, prevents complications, and supports women's reproductive autonomy and agency.

It is important to continue advocating for and working towards ensuring that all women have the right to access safe and legal abortion services, without facing barriers or stigma, and with the necessary support from healthcare systems and providers.

Current scenario: This article highlights important issues related to unintended pregnancies, unsafe abortions, and maternal mortality in India: Below are

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some of the facts related to abortions in the Indian Scenario

1. *Unintended Pregnancies:* Out of the 48.5 million pregnancies that occur annually in India, approximately 44% are unintended. These pregnancies may result from a lack of access to contraception or inadequate knowledge about family planning methods.^[2]
2. *Abortion and Unsafe Abortions:* Of the 16 million unintended pregnancies, about 77% (approximately 12.32 million) result in abortions. However, it is concerning that around 800,000 of these abortions are unsafe. Unsafe abortions are performed without proper medical supervision or in unhygienic conditions, increasing the risk of complications and maternal health problems.
3. *Maternal Mortality:* Among the unsafe abortions in India, 10% result in maternal mortality, meaning approximately 80,000 women may lose their lives due to unsafe abortion practices each year. Maternal mortality refers to the death of a woman during pregnancy, childbirth, or within 42 days after delivery.
4. *Impact of COVID-19 Pandemic:* Between January and June 2020, during the COVID-19 pandemic in India, it is estimated that there were significant increases in unsafe abortions, unintended pregnancies, and maternal deaths. The estimates suggest that an additional 1 million unsafe abortions, 650,000 unintended pregnancies, and 2,600 maternal deaths may have occurred during this period.
5. *Socioeconomic Disparities and Access Barriers:* Poor and illiterate women, those from marginalized castes and religions, and those living in rural regions face greater barriers to accessing safe and legal abortion services. These disparities further increase the risks they face, including the potential for illegal abortions.

These statistics shed light on the challenges faced by women in India regarding unintended pregnancies, unsafe abortions, and maternal health. Addressing these issues requires comprehensive strategies that prioritize access to contraception, education on family planning, safe abortion services, and the empowerment of women in making informed choices about their reproductive health.^[3,4,5]

The Medical Termination of Pregnancy Act, (1971): The act was enacted to provide a legal framework for safe and regulated abortions in India. The Medical Termination of Pregnancy (MTP) Act, of

1971 allowed for the termination of pregnancies up to 20 weeks gestation under specific conditions. These conditions include:

- (1) If the continuance of the pregnancy would pose a risk to the life of the pregnant woman or cause grave injury to her physical or mental health.
- (2) If there is a substantial risk that the child if born, would suffer from physical or mental abnormalities that would be incompatible with a normal life.
- (3) If the pregnancy is a result of contraceptive failure in a married woman or her husband, where they have voluntarily undergone family planning procedures.

This article expresses concern about the issue of unsupervised medical abortion and the potential risks associated with it.

The appropriate time for medical abortion is until 9 weeks or 63 days of gestation. The studies have reported a significant number of patients had self-administered abortion pills, with a considerable portion taking them after the approved period of 63 days of gestation. This indicates a lack of awareness or adherence to the guidelines regarding the proper use of abortion pills.

Unsupervised, self-administered abortion pills in undiagnosed ectopic pregnancy can endanger the life of a woman causing rupture of ectopic, hemoperitoneum and hemorrhagic shock requiring laparotomy and blood transfusions to save the life of a woman.

The common presentation among these patients can be excessive bleeding which can be a serious complication requiring medical intervention. Other complications observed included severe anaemia and shock, which further highlight the risks associated with unsupervised medical abortion. Unsupervised abortion can be dangerous in Rh -ve women and may cause isoimmunization, hypertension, bronchial asthma, cardiac disease, and epilepsy.^[6]

The outcomes of these self-administered abortions are also concerning. A Study reported a majority of patients (62%) had incomplete abortions, indicating that the abortion process was not completed. Additionally, a significant portion (22.5%) experienced failed abortions, where the abortion pills did not effectively terminate the pregnancy. In some cases, incomplete abortion was accompanied by sepsis (7.5%), a severe and potentially life-threatening infection.

Surgical evacuation was required in most

cases (67.5%), indicating the need for medical intervention to complete the abortion process. Some patients (12.5%) also required surgical evacuation along with blood transfusion, emphasizing the potential for severe complications arising from unsupervised medical abortions.^[7] At least 2-3% of women require a blood transfusion, indicating the potential for complications even when following the approved methods.

The common methods adopted for abortion as per various studies were pills followed by Ayurvedic medicine, homemade concoctions (Kahva, papaya, chilli), vaginal insertion of metal sticks/ herbs and external massage. Interestingly, most do not seek advice from a medical person but the source of advice, as noted in various studies, is either a family member, friend, village quack, nurse, Anganwadi worker, local doctor or chemist.^[8]

Access to abortion services can be impeded by various barriers, which can vary depending on the country or region. Here are some common reasons why women may face difficulties in accessing abortion services:^[9]

1. *Legal Restrictions:* Restrictive laws and policies surrounding abortion can create significant barriers for women. In some places, abortion may be entirely illegal or highly regulated, limiting access to safe and legal services but in India, laws are liberal which allows easy access to the services.
2. *Stigma and Discrimination:* Stigma surrounding abortion can deter women from seeking services due to fear of judgment, social ostracization, or discrimination. This stigma can be perpetuated by cultural, religious, or societal norms.
3. *Limited Availability of Services:* Inadequate provision of abortion services, particularly in rural or remote areas, can make it challenging for women to access the care they need. This includes a lack of trained healthcare providers, facilities, or equipment to perform abortions.
4. *Financial Constraints:* Economic barriers, such as the cost of the procedure, transportation, accommodation, and time off work, can make accessing abortion services unaffordable for many women, particularly those from low-income backgrounds.
5. *Legal and Administrative Hurdles:* Requirements, such as long waiting periods, mandatory counselling, parental consent requirements for minors, or mandatory ultrasound examinations or blood tests can create additional barriers and may be barriers to accessing abortion services.

6. *Lack of Information and Education:* Limited knowledge about available abortion services, the legal framework, and women's reproductive rights can prevent women from seeking appropriate care. Insufficient sexual education and limited access to comprehensive information about contraception and pregnancy options can also contribute to barriers.
7. *Provider Refusals and Conscientious Objections:* Some healthcare providers may refuse to provide abortion services due to personal or religious beliefs. This can limit the availability of services and create barriers for women seeking care.

The perception that medical abortions are extremely safe even in the hands of untrained personnel can be misleading and potentially dangerous. While medical abortions using mifepristone and misoprostol have been proven to be safe and effective when used appropriately and under medical supervision, it is crucial to emphasize the importance of proper training and supervision in the administration of these medications.^[10,11]

The recommendation by the Federation of Obstetrics and Gynaecological Societies of India (FOGSI) for close monitoring of the distribution of drugs used for medical abortion reflects the need to ensure that these medications are accessed and used responsibly. The medical profession and pharmaceutical industry should exercise due diligence in promoting and advocating for the safe usage of these drugs.^[12]

Unsupervised terminations and the over-the-counter dispensing of medication abortion pills can lead to potentially life-threatening complications. Without proper medical supervision, individuals may not receive appropriate counselling, information, or follow-up care, increasing the risk of incomplete abortions, unrecognized complications, or inadequate management of side effects.

It is essential to address the societal perception that medical abortions can be safely undertaken without medical supervision. This can be achieved through comprehensive education and awareness programs that provide accurate information about the risks, benefits, and proper usage of medication abortion. Healthcare providers and organizations should play a pivotal role in disseminating accurate information and promoting responsible use of these medications.

Regulatory measures, such as strict guidelines on the distribution and dispensing of medication abortion pills, can also help ensure that these medications are accessed only through appropriate

channels and under medical supervision. These regulations should strike a balance between ensuring access to safe abortion care and mitigating the risks associated with unsupervised medical abortions.^[13,14]

By emphasizing the importance of trained personnel, medical supervision, and responsible usage, it is possible to address the perception that medical abortions can be safely undertaken without proper guidance. This approach promotes the well-being and safety of individuals seeking abortions while ensuring that they receive the necessary support, information, and healthcare services throughout the process.^[15,16,17]

Additionally, it is essential to assess the extent to which healthcare providers are trained according to the World Health Organization (WHO) guidelines on medical abortion procedures and whether these guidelines are being implemented in practice. Understanding the current training practices and identifying any gaps can help improve the quality of care provided by healthcare professionals.

CONCLUSION:

Overall, awareness is needed to address these important aspects and call for action from the government, medical, paramedical personnel, and NGOs to fill the existing knowledge gaps. One of the key steps would be the formation and implementation of strict guidelines on the distribution and dispensing of medication abortion pills, this will help optimize the provision of medical abortion, ensure patient safety, and enhance access to the safe reproductive healthcare option.

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Conflicts of interest

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Review Article

Lead Toxicity in Children: Health Effects, Policies and Recommendations

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ABSTRACT:

Lead is a dangerous element that exists naturally in the Earth's crust. Any kind of lead causes a detrimental response in the human body. It is discharged into the environment during the manufacturing of batteries, foundries, ammunition, lead paint, water pipes, and other manufactured goods. It can enter the body through a variety of pathways, including those in the air, water, soil, food, and dust. Concern is raised since there is no amount of lead that is safe for the human body. The problem persists despite several prevention measures that the state and the federal governments have put in place. This review assesses the effects of lead exposure on children as well as suggested solutions to the issue.

KEY WORDS: lead toxicity; fetal neurotoxicity; lead poisoning; lead damages

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INTRODUCTION:

Lead is a dangerous element that exists naturally in the Earth's crust.^[1]

The environment naturally contains lead. Traces of lead that are naturally present in the environment are not harmful. It is only when lead gets mined and turned into manufactured goods, toxicity sets in.^[2,3] A few examples are petrol, lead based paints and pigments, solder in food cans, ceramic glazes, incineration of lead containing waste, electronic wastes etc. Lead toxicity has recently gained international attention, mainly because lead's toxicity potential never declines, and it is persistent in the environment.^[3] As per *Centers for Disease Control and Prevention* (CDC), the environment around a child is lead filled. Children can be exposed to lead from a variety of sources, including gasoline, solder, water pipes, consumer goods, artificial grass, confectionery, traditional medicine, fine jewelry, and toys.^[4]

Children in the US have recently been exposed

to lead through a variety of unique means. The various sources include lead-containing children's jewelry, Mexican tamarind candy and candy wrappers, food served on imported ceramic bowls and pitchers, breathing lead fumes from melting for fishing weights. Interestingly, there has been a case where the mother and the child were exposed to lead from the bullet in the mother's spine because she had been shot fifteen years earlier.^[5]

Lead exposure and child health effects:

1. Intellectual disability: Lead exposure is associated with intellectual disability. Lead exposure in children with maximum blood lead levels 7.5 µg/dl is connected with intellectual impairments, claims a global pooled investigation by Lanphear et al. They gathered the information from 1,333 children who participated in seven international population-based longitudinal cohort studies, followed from birth or

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infancy until 5-10 years of age in order to examine the relationship between test intellectual scores and blood lead concentration for children with maximum blood lead levels of 10 µg/dl. The full-scale Intelligence Quotient (IQ) score served as the key outcome indicator. 103 (8%) children had a maximal blood lead concentration <7.5 µg/dl and 244 (18%) of the children had maximum blood lead concentrations below 10 µg/dl.^[6] When the confounders were taken into account, it was discovered that the blood lead levels and IQ score had an inverse connection. There was a notable 6.9 decline in IQ [95% confidence interval (CI), 4.2-9.4] with an increase in blood lead levels from 2.4 to 30 µg/dl. A rise in blood lead levels was linked to a decline in IQ.^[6]

2. Fetal Neurotoxicity: There are few other factors that also expose children to lead. For example, lead in maternal bones can be linked to fetal neurotoxicity.^[7] As per the study by Gomaa et al, a 2-fold increase in cord blood lead level (e.g. from 5 to 10 µg/dl) was associated with a 3.1-point decrement in Major Depression Inventory (MDI) score.^[7]

3. Fetal death and low birth weight: Lead-contaminated drinking water is linked to fetal mortality and poor birth weight.^[8] Fetal fatalities reached their maximum point in 2001, when water lead levels were at their highest, and then began to decline after 2004 when various public health actions were put into place, according to an ecological research conducted during the Washington DC lead crisis.^[8]

4. Congenital lead poisoning: Lead can enter an infant's system through the feto-maternal circulation, which can result in congenital problems in infants, or it can enter an infant's system through breast milk.^[9] If the mother has chronic lead toxicity, breastfeeding is one of the other keyways that babies become affected.^[9]

Existing solutions proposed by public health agencies:

Several federal laws such as Environmental Protection Agency (EPA) Lead Renovation, Repair and Painting Program Rule (RRP) requires that certified home renovators be employed to renovate homes that were built before 1978 to replace lead-based paints; The Water Infrastructure Improvements for the Nation (WIIN) Act gave HHS agencies the go-

ahead to establish the required infrastructure to promote lead poisoning prevention measures, and to take actions to support Flint, Michigan in its recovery.^[10]

As per CDC's Blood lead screening policy, all children aged nine months to six years should be screened for lead. However, screening should focus on children who have particular risk factors, such as those who live in inadequate housing or come from low-income households.^[10]

At the state level, Surveillance programs are in place to track the State lead poisoning prevention status and the database is named as Lead Statutes database.^[10]

RECOMMENDATIONS:

Despite all these measures and policies at state and federal levels the lead toxicity in children still prevails. Following measures can be implemented to solve this problem. Policies by FDA to reduce the amount of lead in food and household goods, guidelines to lower airborne lead emissions can be undertaken.^[11] Establishing the surveillance program to identify and address the sources of lead exposure in children similar to that of adults. Implementing policies to provide affordable coverage to ensure that children with elevated blood lead levels have access to developmental and neuropsychological evaluations as well as appropriate, high-quality programs. In addition, increasing public accessibility to local data and artificial intelligence can also be used for surveillance to keep a check in the population.^[12]

CONCLUSION:

Much has been learned about the neurodevelopmental and behavioral effects of lead poisoning in children in recent years. Lead damages the brain and central nervous system at high levels of acute exposure, resulting in unconsciousness, convulsions, and even death. A child who has acute or chronic poisoning is usually left with mental impairment and behavioral problems even if they survive. Lead does not currently have any known safe concentrations. We only know that it has detrimental impact on children's health, notably their mental development, even at extremely low concentrations like 5 µg/dl. Even though several policies are in place to prevent and monitor lead exposures in children, but more effective policies need to be implemented such as complete ban of lead in consumer goods, and a better surveillance program to track exposures. So that children can live a

healthy life free of lead.

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Review Article

Dietary Immunomodulators - An Organic Boom in the Management of Chronic Diseases

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ABSTRACT:

Immunology involves all the defence mechanisms occurring in the body after the invasion of any infectious agent and the ability to resist this infection. The micronutrients like essential proteins, essential amino acids, vitamins (A, B6, B12, C, D, E and folic acid), fatty acids, minerals (iron, selenium, zinc and copper) and certain phytochemicals are of prime importance towards healthy immune system. In addition to these nutritional components, intestinal microflora and certain bacteria (probiotic bacteria) also play an important role in the modulation of healthy immune system. There is an ongoing trend of usage of immunomodulators to combat various chronic diseases like viral diseases, cancers, inflammatory and autoimmune diseases. This review focuses on various immunomodulators available in daily dietary meals, its positive and negative effects on immune system and its role in management of chronic illness as an adjunct to other modalities to achieve positive health benefits with minimal side effects.

KEY WORDS: cancer; immunity; immunomodulator; organic food; probiotics; vitamins

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INTRODUCTION:

Nutrition plays an important role in modulating immune functions. The immune system needs adequate supply of nutrients to function properly. Immune functions are indispensable for defending the body against attack by pathogens or cancer cells, and therefore it plays a pivotal role in the maintenance of health. The immune functions are disturbed by malnutrition, aging, physical and mental stress or undesirable lifestyle. Therefore, the ingestion of foods with immune-modulating activities is considered an efficient way to prevent immune functions from declining and reduce the risk of infection or malignancy. Food-derived substances can

modulate either innate or acquired immunity.

Several studies have shown that the improvement of depressed immune functions by ingesting foods reduced infection rates and mitigated the severity of infectious disease.^[1] Therefore; food substances that are capable of enhancing the immune responses of cancer patients with disturbed/compromised immune functions are valuable. The proliferation and metastasis of cancer cells accelerate when immune functions are disturbed. It has been found that patients with malignancy have lower Natural Killer (NK) cell activity than healthy controls and such persons are subject to higher rates of cancer incidence, metastasis and aggravation of cancer.^[2]

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Mechanism of action of dietary immunomodulators on immune functions:

1. Basic nutrients-

Fatty acids: Polyunsaturated fatty acids (PUFA), are important regulators of several cellular functions, including those related to inflammation and immunity.^[3] Maternal fish oil supplement modifies the balance between cellular n-3 and n-6 PUFAs within the foetus and has the capacity to influence neonatal immune function. It also has potential clinical benefits in reducing the risk of allergic disease.^[4] Dietary supplementation with fish oil in anti-inflammatory doses inhibits prostaglandin E2 (PGE2) synthesis by stimulating peripheral blood monocytes.^[5] This provides a mechanistic basis for the reduction in take of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). The onset of action of fish oil is being evident at 12 weeks after commencement. There is no direct dose-by-dose effect as with the analgesic action of NSAIDs. Clinical benefits have been observed to last up to 6 weeks after discontinuing therapy.^[6] There is evidence that PUFAs regulate the expression of genes for cytokines, adhesion molecules, inducible nitric oxide synthase and other inflammatory proteins.^[7] Since the expression of many of these genes is regulated by the transcription factor, nuclear factor kappa B (NFkB), these observations suggest that PUFAs might affect the activity of this transcription factor.^[8]

Aminoacids:

Arginine is a semi-essential amino acid in mammals. While dietary arginine is not an absolute requirement under normal conditions, it can become essential at times of growth and metabolic stress, such as following trauma, sepsis or burn injuries.^[9] Arginine is required for the normal growth and proliferation of lymphocytes in vitro.^[10] Supplemental arginine also benefits the innate immune response, with increase in macrophage and natural killer cell cytotoxicity.^[11] In the setting of protein-calorie malnutrition and tumour inoculation, supplemental arginine (1% by weight) reduced the growth rate of the immunogenic neuroblastoma C1300 and improved host survival compared with glycine-supplemented controls.^[12] Glutamine is another amino acid having high rate of utilization by neutrophils, macrophages and lymphocytes and its levels get elevated in cellular challenging situations suggesting its importance for functioning of these cells to mount an efficient immune response.^[3]

Carbohydrates:

Dietary monosaccharides like glucose and fructose

and disaccharides like sucrose and lactose^[13] and lectins have capacity to interfere with bacterial and viral attachment to epithelial cell surfaces within the alimentary canal, as they contain mucosal immunoglobulin, secretory IgA. Thus within the alimentary canal, IgA, lectins, bacteria, viruses and mucous membrane exist within a delicate equilibrium which potentially may be perturbed by dietary saccharides.^[14]

2. Micronutrients-Vitamins

Vitamin A:

Vitamin A plays a role in the maintenance of mucosal surfaces, in the generation of antibody responses, in haematopoiesis and in the function of T and B lymphocytes, NK cells and neutrophils.^[15] The influence of vitamin A and its metabolites on different aspects of immune function is attributed to its actions as modulators of gene transcription.^[16] Vitamin A supplementation reduces the morbidity and mortality from measles and diarrhoeal diseases in infants and children in developing countries.^[17,18] Periodic high-dose vitamin A supplementation seems to reduce both morbidity among children born to HIV-infected mothers^[19] and diarrhoeal disease morbidity in HIV infected children.^[20]

Role of Vitamin A in oral diseases:

Vitamin A plays an important role in management of oral leukoplakia and oral submucous fibrosis. It also maintains immune defence responses, oral epithelial integrity, bone growth, normal cell development, avoids keratinisation of mucous forming cells, allows cell differentiation, stimulates osteoclasts and permits normal tooth spacing.^[21]

Vitamin C:

It has an inhibitory effect on the expression of pro-inflammatory cytokines such as interleukin (IL)-6 and tumour necrosis factor alpha (TNF- α) in adult whole blood cells. It has been postulated that vitamin C might be an interesting compound for modulation of an overexuberant immune response.^[22]

Role of Vitamin C in oral diseases:

Study conducted by R. Guruprasad et. al in 2014 evaluated the correlation between Serum Vitamin C and Iron levels in OSMF patients. The level of Serum Vitamin-C and Iron was significantly decreased in OSMF patients when compared to controls. They concluded, that the chemical, thermal and/or mechanical factors associated with the use of areca nut may act in conjunction with the Vitamin C and Iron

deficiency leading to the development of OSMF. Therapeutic substitution of Vitamin C and Iron may be recommended in the management of OSMF.^[23]

A study conducted by Supriya Bhat et al, in 2017 aimed to estimate the detoxification status of serum and saliva by assessing the serum and salivary Vitamin C in oral potentially malignant disorders and oral cancer. A total of 90 subjects, 30 subjects with oral potentially malignant disorders, 30 subjects with oral cancer, and 30 healthy subjects (controls) were included in the study. The study concluded that the mean serum and salivary Vitamin C levels were decreased significantly in potentially malignant disorders and oral cancer when compared to healthy subjects.^[24]

Vitamin E:

It is the most effective chain breaking, lipid-soluble antioxidant in cell membranes and plays a major role in maintaining cell membrane integrity by limiting lipid peroxidation by reactive oxygen species (ROS).^[25] It can influence a variety of inflammatory processes by inhibiting the activity of NFκB, required for maximal transcription of many proteins involved in inflammatory responses, including several cytokines, such as IL-1β, IL-2 and TNF-α.^[26] Vitamin E supplementation in diet enhances antibody production, lymphocyte proliferation, NK-cell activity, and macrophage phagocytosis.^[27]

Carotenoids:

The bright red, yellow and orange pigments in various fruits and vegetables are attributed to Carotenoids. The prevalent ones in the human diet include beta-carotene, alpha-carotene, beta cryptoxanthin, lycopene, lutein, and zeaxanthin, having provitamin A activity, and are converted to retinol, the active form of vitamin A, in the body.^[22] Lycopene has been shown to have high singlet oxygen quenching capability and has been associated with anti-tumour promoting activities in various tissues in animal studies. Association between lycopene intake, primarily from tomato products is associated with reduced risk for cancers at all sites.^[28] Epidemiological studies have found significant associations between higher plasma beta cryptoxanthin and a reduced risk for gastric adenocarcinomas and lung cancer in men.^[29,30] Consuming a carotenoid-rich diet may be one of the ways for reducing the risk of cancer.^[22]

Role of Carotene in oral diseases:

Lycopene has been hypothesized to prevent carcinogenesis and atherogenesis by protecting critical

cellular biomolecules, including lipids, lipoproteins, proteins, and DNA. Dosage of 4.8 mg/day orally for 3 months leads to the reversal of dysplastic changes in leukoplakia and dosage of 16 mg/day leads to substantial increase in the mouth opening in oral submucous fibrosis. In vitro studies have shown lycopene to be twice as potent as β-carotene and ten times that of α-tocopherol in terms of its singlet oxygen quenching ability.

Thiamine:

Thiamine is a water-soluble Vitamin B.^[22] Several case control studies have reported significant associations between thiamine intake and reduced risk of several cancers including colon, and colorectal.^[31,32]

Folate:

Folate is a water-soluble vitamin B found naturally in certain citrus foods called food folate, and the synthetic form added to fortified foods and supplements, called folic acid. Several studies hypothesized that folate supplementation is associated with a decreased rate of infection, positive effects on blastogenic response and proliferation of T lymphocytes, enhanced delayed-type hypersensitivity response, enhanced phagocytosis, and immunoglobulin production. It appears to have no effect on NK cell function. Immunologically folate deficiency are likely caused by defects in DNA and RNA synthesis or methyl metabolism.^[22]

Vitamin B-6:

It affects immune function as it plays a role in the formation of the amino acid cysteine, an important precursor in glutathione, which is closely associated with lymphocyte proliferation.^[33] Its potential mechanism to reduce cancer risk includes reducing the disruption of DNA synthesis, repair, and methylation associated with inadequate intake.^[25] Also it may reduce cancer cell proliferation and oxidative stress, suppress nitric oxide, or have antiangiogenic properties.^[34]

Minerals:

Zinc-

High content of Zinc (Zn) is present in animal protein food.^[35] Zn plays a role in T lymphocyte activation and signal transduction. Zn has been implicated in the non-covalent interaction of the cytoplasmic tails of CD4 and CD8 with the tyrosine kinase p56lck, an essential process in the early steps of T-cell activation.^[36] Zn stimulates autophosphorylation of tyrosine residues by p56lck and subsequent

phosphorylation of the T-cell receptor complex involving CD45.^[37] Zn-deficient children with acrodermatitis enteropathica (AE) have reduced numbers of lymphocytes, particularly T-cells, in the blood and peripheral lymphoid tissues. Decreased CD4+/CD8+ cell ratios are also seen. T-cell responses, such as proliferation in response to mitogens, cytotoxicity and delayed-type hypersensitivity (DTH), natural killer cell activity and chemotactic response of the monocytes are suppressed.^[38,39] Controlled trials of Zn supplementation demonstrated a reduction in the incidence and duration of acute and chronic diarrhoea by 25-30 %, and in the incidence of pneumonia by up to 50%.^[40]

Role of Zinc in SARS-CoV 2:

It is known that zinc (Zn) possesses a variety of direct and indirect antiviral properties. Administration of Zn supplement has a potential to enhance antiviral immunity, both innate and humoral, and to restore depleted immune cell function or to improve normal immune cell function, particularly in immunocompromised or elderly patients. Zn may also act in a synergistic manner when co-administered with the standard antiviral therapy, as was demonstrated in patients with hepatitis C, HIV, and SARS-CoV-1. Zn may also protect or stabilize the cell membrane that blocks the virus entry into the cell. It also inhibits viral replication by alteration of the proteolytic processing of replicase polyproteins and RNA-dependent RNA polymerase (RdRp) in rhinoviruses, HCV, and influenza virus, and diminish the RNA-synthesizing activity of nidoviruses, that belongs to SARS-CoV-2. Therefore, it has been hypothesized that Zn supplements may have potential benefit in prophylaxis and treatment of COVID-19.^[41]

Iron:

It is an essential nutrient for cells because of its role as a cofactor for enzymes in the mitochondrial respiratory chain and oxidative phosphorylation, in the citric acid cycle (aconitase), and in DNA synthesis (ribonucleotide reductase).^[42] Iron interferes with cytokine activities and the cell-mediated immune effector mechanisms of macrophages, thus altering the immune response toward invading pathogens.^[43] One central mechanism responsible for this is a direct inhibitory effect of iron on IFN- γ activity. Iron loading of macrophages results in an inhibition of IFN-mediated pathways in macrophages, such as formation of the pro-inflammatory cytokine, TNF- α and expression of MHC class II antigen.^[44] Part of this effect results from the reduced formation of nitric

oxide (NO) in the presence of iron. This is important, because NO is an essential effector molecule produced by macrophages to fight infectious pathogens and tumour cells.^[45] Iron blocks the transcription of inducible NO-synthase (iNOS or NOSII), the enzyme responsible for cytokine inducible high-output formation of NO by hepatocytes or macrophages.^[46] The inhibitory effect of iron toward IFN- γ activity also affects the Th1/Th2 balance, with Th1 effector function being weakened, whereas Th2-mediated cytokine production and function, such as IL-4 activity is increased which is unfavourable during a malignant disease or an acute infection. B cells are not prominently affected by iron homeostatic changes, while NK cells are sensitive to iron homeostatic imbalances with impaired proliferation in iron deficiency and overload.^[44]

Selenium:

Various milled wheat and corn products may contain 70% or more of selenium. Cooking appears to result in little significant loss of selenium in most foods, but dry heating of cereals may result in significant reductions in their original selenium content.^[47] A major immune stimulatory effect of Selenium is by up-regulation of expression of the α and β subunits of the IL-2 receptor, which are expressed on many immune cells and notably on T and B lymphocytes. This increases the ability of these cells to respond to IL-2. Stimulation with IL-2 from activated CD4+ T-helper cells potentiates the cytotoxicity of killer cells, increases numbers of lymphocytes, promotes antibody production by B lymphocytes and improves the responsiveness of immature bone marrow cells to other cytokines in order to produce immune-cell precursors.^[48] Supplementation with Selenium appears to reverse the age-related decline in NK cell function in elderly individuals. The loss of NK-cell activity is a means by which cancer cells may evade immune-mediated destruction.^[49]

Role of Selenium in oral diseases:

Oral lichen planus (OLP) is a chronic disease with immune mediated pathogenesis. Selenium (Se), an antioxidant, plays a role in modulating immunity. Passant O. Qataya et al, in January, 2020 conducted a randomized control clinical trial to evaluate two forms of Selenium (*novel* topical hydrogel and oral capsules), solely, in treating erosive OLP based on clinical evaluation and salivary oxidative stress markers. Patients were allocated into one of three groups: group I, topical corticosteroids; group II, topical Selenium; and group III, systemic Selenium.

Treatment lasted for 6 weeks; patients were clinically evaluated at baseline, 6 and 12 weeks. Patients in all the groups showed significant reduction in symptoms after the treatment. However, group II had significantly lower pain scores at the end of 12 weeks compared to the other groups.^[50]

Probiotics:

Under normal circumstances, the resident gut bacteria cause neither pathogenicity nor inflammation in the host, but instead contribute to health maintenance, forming a barrier layer against colonization by pathogens and aiding in nutrient digestion and assimilation.^[51] The resident intestinal microflora plays important physiological roles like deconjugating potentially damaging oxidative metabolites and toxins in the gut; degrading potentially allergenic food proteins; regulating cholesterol and triglyceride uptake; increasing vitamin biosynthesis and providing immunosurveillance signals to limit intestinal-tract inflammation. Thus, a stable, properly functioning and active intestinal tract microflora is essential to the continuance of human health. Among the most predominant microbes in the human intestinal tract are the Gram positive lactic acid-producing genera *Lactobacillus* and *Bifidobacterium*. *Lactobacilli* and *bifidobacteria* are also common fermentative microbes in yoghurt and cheese.^[52] Certain strains of probiotic lactic acid bacteria (LAB) can prime peritoneal macrophage populations for enhanced phagocytosis, lysosomal enzyme production and free radical oxidant production.^[53] In human studies, consumption of *Lactobacillus rhamnosus* (strain HN001) or *Bifidobacterium lactis* (strain HN019) has been demonstrated to up-regulate peripheral blood NKcell-mediated cytotoxicity against tumour cells.^[54] In addition to anti-allergy immunoregulation, probiotics also combat inflammatory diseases. Evidences demonstrate that dietary consumption of immunoregulating LAB might assist in combating autoimmune diseases, including juvenile chronic arthritis.^[55] The potential use of probiotics to augment the routine immune signalling events of the gut microflora, as a means of restoring vital anti-inflammatory immunoregulatory control mechanisms is a promising means of combating inflammatory bowel disease.^[56]

Prebiotics:

These are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one species or a limited number of species of bacteria in the colon.^[57] Unlike

probiotics, that introduce exogenous bacteria into the human colon, prebiotics stimulate the preferential growth of a limited number of health-promoting commensal flora already residing in the colon.^[58] Dietary raffinose suppresses serum immunoglobulin E response through suppression of Th2-type immune response against oral antigen in the lymphoid organs located in or near the intestine.^[59]

Food beneficial to the immune system:

Epidemiological and experimental studies have demonstrated a negative correlation between the deficiency of diets rich in fruits, and vegetables and the risks for chronic diseases, such as chronic inflammation and cancers.^[60] Therefore, adequate supplementation with fruits and vegetables might play an important role in the control of acute and chronic diseases via immuno-modulation. Dark coloured fruits and vegetables have potential in stimulating Th1/Th2 cytokine secretions.^[61]

Strawberries, red onions, peppers and spinach:

Strawberry and red onions demonstrate an immunomodulatory potential via stimulating splenocyte proliferation. The immuno-modulatory components in these fruits are correlated with phenolics, including flavonoids.^[61]

Apples:

It one of the main sources of dietary flavonoids. Apple extracts can significantly inhibit the TNF-alpha-induced NF-kappa B activation at a dose of 5 mg/ml.^[62]

Carrots, celery and parsley:

Non-toxic doses (20 µg/ml) of these foods and their related ingredients might act to affect health as immune-stimulating agents, i.e. directly enhancing lymphocyte activation and/or secreting multipotent cytokine IFN-γ.^[63]

Cruciferous vegetables:

Increased intake of cabbage, cauliflower, broccoli, Brussels sprouts, watercress, and mustard greens, is associated with a decreased risk of several cancers in human population.^[64] This is achieved by alterations in the activities of metabolic enzymes that result in reduced carcinogenicity of dietary or environmental carcinogens, reduction of oxidative DNA damage levels in human lymphocytes in increased oxidative stress conditions.^[65,66,67]

Tomato:

Epidemiological and experimental data suggest that an increased intake of tomato products can reduce the risk of cancers, especially prostate, colon, and oral cancer. Several evidence suggest that tomato constituents, such as lycopene, affect immune functions modulation and antioxidant activity.^[68]

Garlic:

Allicin is the active ingredient of freshly crushed garlic. It significantly inhibits the secretion of IL-8, INF- γ -inducible protein of 10 kD (IP-10), monokine induced by INF- γ (MIG) and IL-1b from intestinal epithelial cells. These cyto-and chemokines play an important role in the pathogenesis of inflammatory bowel diseases. Therefore, local application of allicin may serve as a potential immune-modulating therapy in inflammatory bowel diseases.^[69] Meta-analyses revealed that increased garlic consumption diminished the risk of stomach and colorectal cancers.^[70]

Soybeans:

It possesses several traditional phytonutrients and several bioactive phytochemicals including flavonoids and saponins, with variety of potential health benefits, like anti-inflammatory, anti-oxidative, anti-mutagenic and anticarcinogenic effects.^[71-74]

Cereals (rice and wheat):

Lipopolysaccharide (LPS) or LPS-like components associated with cereal grains play a major role in IL-10 production from Peripheral Blood Mononuclear Cells. The ability of various food products to induce IL-10 production did not always correlate with the concentrations of LPS in their extracts. Therefore LPS or LPS mimicking molecules likely work in concert with other immune stimulatory or immune regulatory molecules in the cereals, such as carbohydrate polymers or lectins, to induce robust IL-10 production. This immune modulatory effect might explain why most individuals who are at genetic risk for celiac disease do not acquire celiac disease or inflammatory bowel disease.^[75]

Mushrooms:

Mushroom proteins are potent immune activators and tumour cell growth inhibitors, mediating their effects by regulating cytokine secretion and proliferation, and are mitogens and immune modulators with therapeutic potential.^[76]

Honey:

The most promising bioactive compounds found in honey products are the proteins of royal jelly.^[77] These proteins may have physiological functions as suppressors of allergic reactions, as well.^[78]

Dairy products:

Yogurt is one of the best-known foods that contain probiotics. In human studies, cytokine production, phagocytic activity, antibody production, T cell function, and NK cell activity were shown to increase with yogurt consumption or when cells were exposed to LAB in vitro. There is evidence that yogurt-induced immune enhancement is associated with a lowered incidence of cancer, GI disorders, and allergic symptoms.^[79] Denatured and native whey protein, both of which have remarkably higher cysteine contents than do other common edible proteins, may contribute to the immunostimulatory effects of yogurt. Cysteine is a rate-limiting component in the biosynthesis of glutathione. Glutathione is important for detoxification of endogenous and exogenous carcinogens and free radicals and in regulation of immune functions.^[80]

Cheese constitutes another family of milk derived fermented products and its consumption exerts a stimulatory effect on immune system functions.^[81] In addition to the bacterial cell components, the immunomodulatory effect of cheese could also result from non-bacterial components such as peptides which in a lyophilized extract of Gouda cheese suppresses proliferation of cultured human peripheral blood lymphocytes in vitro and induces apoptosis of human promyelocytic leukaemia cells.^[82]

Detrimental effects of food on the immune system:

Food allergies are caused by immunologic pathways that include activation of effector cells (mast cells and basophils) through food specific immunoglobulin E (IgE) antibodies (IgE-mediated food allergy), cell-mediated reactions resulting in subacute or chronic inflammation (non-IgE mediated food allergies), or combined pathways. IgE-mediated reactions occur rapidly (within seconds to minutes) after ingestion of the offending foods. Rarely, reactions take up to two hours and beyond to occur.^[83] Manifestations of IgE-mediated food allergy include acute urticaria and angioedema, rhinoconjunctivitis, asthma, nausea, vomiting abdominal cramps and diarrhoea. However, generalized urticaria and anaphylaxis can also occur.^[84] The oral allergy syndrome is an IgE mediated hypersensitivity is

considered as a form of contact allergy to certain (usually fresh) fruits, nuts and vegetables and seen in up to 50% of patients with allergic rhinitis to pollen.^[85] Symptoms are mainly confined to oropharynx, and include the rapid onset of pruritus involving the lips, mouth and/or pharynx. Mild swelling of the lips is common. Symptoms generally subside within minutes after ingestion ceases. However, progression to systemic symptoms is thought to occur in approximately 10% of patients.^[83] Over-activation of the immune system can lead to harmful effects such as chronic inflammation or autoimmune diseases. Sometimes, the body begins to manufacture T cells and antibodies directed against its own cells and organs. Some individuals develop an exaggerated immune response to food through developing food allergy which may be IgE mediated, non-IgE mediated, or mixed.

CONCLUSION:

Nutrition plays an important role in the modulation of immune function and the various food components like proteins, vitamins, fatty acids, minerals, phytochemicals and probiotics play an important role in the modulation of immune function which is directly associated with immunological tolerance toward diseases. Supplementation with single or multiple micronutrients may enhance immune functions even in healthy individuals. On the other hand, excess amounts of some nutrients may impair immune functions.

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Conflicts of interest

There are no conflicts of interest.

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Case Report

Suture Less Gingival Augmentation Using Gingival Unit Transfer – A Case Report

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ABSTRACT:

The use of root coverage procedures to treat gingival recession defects, a common periodontal condition, is an important aspect of periodontal regenerative therapy. The synergistic relationship between vascular configuration and involved tissues is the most important factor in soft tissue graft success.

The present case reports the clinical effectiveness of Gingival Unit Graft (GUG) for the management of Miller's class III gingival recession. Clinical parameters like Probing depth, recession depth, keratinized tissue width and clinical attachment level were measured at baseline and postoperative 6 months. Percentage of defect coverage was evaluated at postoperative 6 months.

Healing was uneventful and 3mm root coverage was observed with 1mm residual recession and increase in keratinized gingiva after 6 months follow up.

Free soft tissue autografts such as gingival unit transfers can be used along with bio-adhesives such as cyanoacrylates for predictable results in the management of recession defects.

KEYWORDS: gingival recession, keratinized gingiva, free soft tissue autograft, cyanoacrylates

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INTRODUCTION:

Gingival recession is defined as the oral exposure of the root surface caused by a displacement of the gingival margin apical to the cemento-enamel junction, and it is frequently associated with dental aesthetic deterioration. The use of root coverage procedures to treat gingival recession defects, a frequently reported periodontal condition, is an essential consideration of periodontal regenerative therapy. Many surgical procedures can be used to treat recession defects, including free gingival grafts, connective tissue grafts, acellular dermal matrix grafts, various pedicle flaps, combinations of these pedicle flaps and graft techniques, and guided tissue

regeneration. The literature review reveals varying success rates and predictability with these surgical procedures^[1].

The synergistic relationship between vascular configuration and involved tissues is the most important factor in soft tissue graft success^[2]. The graft's vascular characteristics are most likely important for rapid anastomosis of the recipient site's capillaries with injured graft vessels^[3]. Fine blood vessels form a network in the gingival sulcus, and capillaries have numerous anastomoses^[4]. Because the gingiva's vascular plexus is rich in horizontal anastomoses that perfuse the marginal zone, marginal and interdental gingival tissues could be used to

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benefit from improved blood perfusion of the recipient site, improving graft survival^[5]. As a result, the supracrestal part of healthy gingiva that included marginal and papillary tissues is thought to be the only soft tissue with a free marginal portion naturally established to survive and function over a vascular root surface^[3]. Other research has found that healthy gingiva has substantially different vascular patterns in the marginal, attached, and interdental gingiva^[6].

Site-specific donor tissue is presumed to have improved potential for function and aesthetic acceptance at recipient sites in soft tissue graft procedures. Clinically, using a site-specific Gingival unit graft placed on a traditionally prepared recipient site results in predictable root coverage. Gingival Unit Transfer (GUT) is a variation of Free Gingival Graft (FGG) that includes marginal gingiva and papillae in the traditional palatal tissue graft with a vascular supply that matches the recipient site intimately^[2].

Sutures have long been demonstrated to provide appropriate wound closure while having a low rate of dehiscence. However, their use in root coverage has some drawbacks. The main disadvantage is that it increases surgical time. It also traumatizes tissues, affecting vascularization and increasing the risk of flap tearing^[7]. Furthermore, based on the material, it can pinch and irritate patients or promote plaque accumulation, enhancing susceptibility to infection^[8].

Tissue adhesives, are biocompatible agents that, when applied to skin or mucosa, demonstrate a resistance to wound dehiscence via attractive forces between the tissue and adhesive molecules, and are an alternative to sutures. Because cyanoacrylates are biocompatible, biodegradable, hemostatic, and have a long half-life, they are an excellent adhesive agent for the oral environment^[9].

This adhesive was used as an adjunct to intraoral/extrabuccal wound suture by Nevins et al.^[10] because it can be implemented faster, prevents ischemia, and improves haemostasis. In general, a longer operative time has been linked to increased bacterial exposure, a greater amount of anaesthesia, and a higher morbidity rate. In this study, using cyanoacrylate tissue adhesive instead of suture resulted in a significant decrease in operative time (mean of 4.5 min) (mean of 8 min). According to Stavropoulou et al.^[11], the duration of treatment was 3-fold shorter with cyanoacrylate than it does with conventional suture.

The present case report describes utilization of gingival unit graft for gingival augmentation and stabilization of graft over recipient site using cyanoacrylates.



Figure 1



Figure 2

Figure 1&2: Recession of 4mm with inadequate keratinized gingiva & shallow vestibule.

CASE REPORT

A 24-year-old female patient came to the department of periodontology with a complaint of receding gums in the lower front tooth region & inability to maintain oral hygiene. Patient has a history of orthodontic treatment in her previous dental visit. Patient gives no relevant medical & family history. On intra-oral examination, mild supragingival calculus was present. Gingiva exhibits soft & edematous with rounded margins in relation to tooth #41. A 3.3 mm wide & 4 mm deep Miller class III gingival recession defect with inadequate keratinized gingiva was found on mandibular right central incisor. The probing depth was 1 mm and the CAL was 5 mm (Figure 1 & Figure 2). Patient also presented with mucogingival problems like shallow vestibule and frenal pull in relation to tooth #41. Patient was diagnosed as chronic generalized gingivitis with localized periodontitis in

relation to tooth #41. Initially phase-1 therapy i.e., full mouth scaling & root planing in relation to tooth #41 was done. After all the confirmed inflammatory signs were reduced after initial phase-1 therapy, Gingival Unit Transfer was planned to reduce the recession & to provide adequate zone of attached gingiva in relation to tooth #41.

Presenting problems in this case report were a 3.3 mm wide & 4 mm deep Miller class III gingival recession int #41, inadequate attached gingiva (tension test positive) which leads to progressing gingival recession, inadequate depth of vestibule (inability to maintain oral hygiene) & frenal pull.



Figure 3: Two divergent vertical bevelled incisions given at recipient site



Figure 4: Recipient site prepared using split thickness dissection showing dehiscence.

METHODOLOGY:

After achieving adequate anesthesia, root planing was done in the exposed portion of the root surface and then irrigated with saline. The recipient site was prepared by giving two vertical beveled incisions that extended apically to adjacent teeth,



Figure 5



Figure 6

Figure 5&6: Gingival unit graft harvested from palatal aspect of #25

3 to 4 mm beyond the mucogingival line, and the surfaces of interdental papillae were removed. The incisions were divergent therefore the recipient site was trapezoidal. At the mucogingival line, vertical incisions were connected by a horizontal incision (Figure 3). A partial thickness dissection was made apical to the alveolar mucosa and care was taken to relieve the frenal attachment along with vestibuloplasty to increase the depth of vestibule. The epithelial surfaces within these incisions were de-epithelialized. After preparation of recipient site, dehiscence was found in relation to tooth #41 (Figure 4). The base of the recipient site was made 5 mm apical to the apical part of the exposed portion of the root surface. The Gingival unit graft was harvested from the palatal aspect of #25 including the marginal gingival tissue and the papillae (Figure 5 & Figure 6). The graft was placed over the recipient site and gentle pressure

was applied for 2-3 minutes for achieving primary fibrin stabilization and for preventing formation of dead space (Figure 7). Graft was stabilized using cyanoacrylates (AMCRYLATE -ISO AMYL 2 CYANOACRYLATE). The donor site (palatal aspect of #25) was also covered with cyanoacrylate (Figure 8 & Figure 9). The operated site was covered using non eugenol periodontal dressing for protection which was removed after 1 week. At the postsurgical care for infection control, patient was advised to rinse twice daily with 0.2% chlorhexidine mouth wash for 3 weeks and asked to avoid brushing and hard chewing at the surgical site. 2 weeks post-operatively, recipient site showed complete healing with 5mm keratinized tissue gain and 1mm residual recession in relation to tooth #41(Figure 10). Patient was recalled at 3 months and at 6 months (Figure 11 & Figure12). The recession defect coverage was stable. The colour match of the graft with the adjoining tissues was acceptable aesthetically.



Figure 7: Graft placed at recipient site & stabilized using cyanoacrylates.



Figure 8: Donor site covered with cyanoacrylate.



Figure 9: Donor site covered with cyanoacrylate.



Figure-10: 1mm residual recession with 5mm keratinized gingiva & Increased depth of vestibule.

DISCUSSION:

Gingival unit transfer is a variation of the Free gingival graft that includes marginal gingiva and interdental papillae in the conventional palatal tissue graft whose vascular supply matches integrally with the recipient site, as described by Allen AL and Cohen DW^[12]. Gingiva has a distinct structure and properties^[13]. The gingival arterioles are oriented apico-coronally. Capillaries form repetitive networks in the marginal gingiva, and several small vessels form loops that extend towards the marginal gingiva. Furthermore, it has also been demonstrated that as gingival vessels extend coronally, their size and number decrease. Thus, in this modified technique, the donor tissue's size, number of vessels, and vascular configuration would better match those of the recipient



Figure 11: 1mm residual recession with 5mm keratinized gingiva & Increased depth of vestibule after 2 weeks.



Figure 12: 6 months post-operative follow-up photograph.

site, providing a desirable aesthetic outcome and tissue blend^[14]. A successful surgery requires wound closure. Inadequate suturing or postsurgical care by the patient could result in complications such as graft loss^[15].

A suture material with good capillary action acts as a wick, delivering serum fluid and bacteria, making it susceptible to complications and surgical failure. The angiographic analysis done immediately just after surgical treatment revealed that the micro-surgically operated sites had better vascularization with a mean percentage of $8.9 \pm 1.9\%$ compared to a macro-surgically treated sites with $8.0 \pm 1.8\%$, respectively. The observed difference provided evidence that a minimally invasive technique may result in less tissue trauma. It is possible that the reduced tissue damage was caused by the sharper and finer surgical blades and finer suture material used

during the microsurgical approach^[16].

Tissue adhesives could help with some of these issues. Tissue adhesives form a strong adherence surface, allowing the graft to be stabilized without perforating the flap and ensuring a better blood supply. Furthermore, they act as a barrier, securing the wound edges and preventing infection or graft detachment, attempting to make surgical techniques more predictable^[9]. It serves as a physical barrier over the donor site, promoting faster wound healing and increasing patient comfort.

As the cyanoacrylate is hard to control, it can get under the graft. Care should be taken while grafting larger areas with free soft tissue grafts, where there are chances for dead space formation which are considered as limitations of cyanoacrylates.

Jenabian et al.^[14] used a split-mouth design to treat 18 bilateral localised recessions (Miller Classes I and II) in nine systemically healthy patients. Gingival Unit Graft (GUG) produced higher aesthetic satisfaction at 1, 3, and 6 months, as well as higher root coverage at 1 month, according to the study. Furthermore, 11% of GUGs had complete coverage at 6 months, as well as a higher healing index and substantial reduction in recession width 3 months after surgery; however, the reduction in vertical recession depth (VRD) on this side was not statistically significant.

Kuru and Yildirim's^[3] study, included 17 patients who were randomly assigned to one of two groups. The authors discovered that the GUG group had greater vertical recession reduction, attachment, and keratinized tissue gain than the second group. Furthermore, the mean proportions of defect coverage in the GUG group were 91.62% and 68.97% in the FGG group ($P<0.05$).

Sibel Kayaalti-Yuksek & Emre Yaprak^[17] compared the usefulness of GUG with connective tissue graft using a randomized split mouth study to verify the clinical parameters and patient scores in gingival recessions. Sixteen patients with bilateral defects were chosen. SCTG or GUG was performed in thirty two defects. Clinical measurements, Recession coverage and patient outcomes were measured at baseline and at 1, 3 and 6 months. They concluded that GUG can be a suitable method for treatment of recession with inadequate Keratinized tissue width.

In a systematic review on comparison of gingival unit transfer and free gingival graft done by Chethana et al^[18], the authors could not prove the superiority of gingival unit transfer over Free Gingival graft. But, GUT showed higher percentage of sites with comprehensive root coverage than FGG.

CONCLUSION:

For predictable results in the management of recession defects, free soft tissue autografts such as gingival unit transfers can be used in conjunction with bio-adhesives such as cyanoacrylates. Although this technique is simple and minimally invasive, considerations such as proper plaque control, root surface biocompatibility, careful surgical manipulation, and tissue thickness have been shown to be critical and may affect the grafting procedure's outcome. Clinical trials are needed to demonstrate the efficacy of this technique in the treatment of class III gingival recession.

Financial Support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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Case Report

Aphthous Ulcer: A Case Report

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ABSTRACT:

Mouth ulcers are quite common in all age groups. They can be linked to several conditions such as simple injury due to acute trauma or due to a sharp tooth or to an autoimmune disease. There are several medications available on the market to treat mouth ulcers. But do we need to prescribe a medication every time? This report presents a case of a 34-year-old male who presented with an ulcer in the palate and was treated without a prescription.

KEYWORDS: aphthous; ulcer; cankersore

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INTRODUCTION:

An internal sore in the mouth known as a mouth ulcer can form anywhere. It is possible that a person can have one or more of these sores, which are typically red, yellow, or white. They can appear on gums, tongue, palate, oral mucosa, inner surface of the lips. They can make eating, drinking, and speaking very painful. They can occur due to bacterial or viral infection, sharing food with an infected person, digestive problems, autoimmune diseases or simply stress.^[1]

A few types of ulcers that a dentist may come across frequently are traumatic ulcers that occur because of minor trauma, stress, or digestive problems. Differential diagnoses may also include contact allergic stomatitis, traumatic fibroma, herpes simplex virus infection (cold sore), and aphthous stomatitis.^[2] Traumatic ulcers are caused by a sharp tooth, sharp edges of the appliance within the mouth or cheek biting.^[2] Ulcers can also be caused due to bacterial or viral infections. Ulcer location can also depict the reason causing it. For example, ulcer caused by herpes simplex viral infection occurs in vermillion border, attached gingiva, hard palate etc.^{[1][1]}

Oral ulcers usually resolve by themselves in

about a week if the cause is removed. Symptomatic relief can be achieved with over-the-counter anesthetic creams/gels and chlorhexidine 0.2% aqueous mouthwash to maintain good hygiene. However, mouth ulcers that last more than two weeks and don't resolve after removing the cause should be referred for biopsy.^[3]

CASE PRESENTATION:

A 34-year-old male came to our clinic with a complaint of sore in his mouth. He never had similar sores of such big size before. On clinical examination we found an ulcer 1 X 2 cm in diameter extending posterior to the molar area on the palate, not crossing the midline. The ulcer had a yellowish white appearance in the center and red erythematous border. The ulcer had been present for 2 days without causing any pain, itching or difficulty in eating. The oral hygiene of the patient was good with mild stains on some teeth. No signs of bleeding gums or tooth decay were evident. Further investigations and body vitals revealed no deviations from the normal. We diagnosed the case to be of aphthous ulcer major due to its size, presentation, and location (Figure).

Since the oral hygiene status of the patient was good and he did not have any signs of pain we

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Figure : Aphthous ulcer located on the left side of the palate.

prescribed non-alcoholic mouth rinse to prevent secondary infection, and warm saline rinses two to three times a day to counter inflammation. The patient returned after 10 days with a completely healed ulcer. The patient was scheduled for further follow-up after three months to check for recurrence.

DISCUSSION:

Break in the oral mucosal lining or erosion is referred to as mouth ulcer.^[9] Even though oral ulcers are not dangerous; they can pose discomfort to the patient. Canker sores can affect about 20% of the population. Since, the etiology is idiopathic, it is believed that they occur due to the activation of the cell mediated immune system.^[7]

They can occur due to the deficiency of vitamins Vit B, folate, or iron.^[1] Other common causes might be sensitivity to certain food items. The most frequent causes include spicy or acidic food, hormonal shifts, or even genetics.^[5] The exact cause of their occurrence is still unknown.^[1] Canker sores can often be painful, causing difficulty in speaking, eating or drinking but are not contagious.^[4] They do not leave a scar on healing.

Unlike this case, major aphthous ulcers might take up to six weeks to heal and they might leave a scar. Herpetiform ulcers, on the other hand, occur in small clusters of pinpoints 10 to 100 sores fusing together to form a large ulcer that is irregular in shape.^[10] However, they also heal within one to two weeks without scarring.^[4] Large aphthous ulcer that have slow healing might also be associated with HIV. Therefore, HIV testing must be carried out while ruling out the cause.^[8] A visit to a doctor is required when the discomfort is hindering day-to-day activities due to severe pain or

discomfort. Even non-painful ulcers that do not heal within 2 weeks should warrant a thorough medical work up.^[6] In most cases anesthetic creams such as orajel can be prescribed for symptomatic relief.

CONCLUSION:

Ulcers can occur due to a variety of reasons and might look concerning. But they are very common and there are a lot of treatment options available for symptomatic relief while they heal on their own. This particular case is a good example to show that if there is no pain or discomfort even a huge ulcer can heal on its own. Taking proper history can help us rule out the cause, which in most cases is unknown for aphthous ulcers.

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Conflicts of interest

There are no conflicts of interest.

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Case Report

Mucormycosis- A Case Report

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ABSTRACT:

Mucormycosis, an aggressive and opportunistic fungal infection caused by Rhizopus sp., Mucor, and Lichtheimia, poses a significant challenge in the post-COVID era. Previously considered a rare occurrence, mucormycosis has witnessed a surge in cases, particularly affecting the nose, paranasal sinuses, and cerebral tissue. These fungal pathogens exhibit a destructive behaviour, eroding small blood vessels and leading to thrombosis, ischemia, and tissue necrosis. Patients with compromised systemic health, such as diabetes mellitus, leukemia, and immunosuppressive therapy, are particularly susceptible to this infection due to impaired immunity.

The various clinical manifestations of mucormycosis are categorized into rhinocerebral, pulmonary, cutaneous, gastrointestinal, and disseminated forms. Within the rhinocerebral form, subdivisions based on the affected tissues further refine the classification like rhino-orbital, rhino-sino-orbital, rhino-orbito-cerebral.

Fungal culture remains a cornerstone for identifying the causative organism, while magnetic resonance imaging is the gold standard for radiological evaluation, offering detailed imaging of the affected regions. Computed tomography scans also play a crucial role in the diagnostic pathway.

With dental practitioners encountering an increasing number of mucormycosis cases, Cone Beam Computed Tomography has emerged as a valuable diagnostic tool. Recent advancements have led to the development of diagnostic criteria based on CBCT findings, aiding in the accurate and timely diagnosis of mucormycosis. We report a case of mucormycosis affecting maxilla highlighting the importance of CBCT in addition to conventional diagnostic methods thereby improving its management and clinical outcome.

KEYWORDS: mucormycosis; COVID-19; CBCT.

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INTRODUCTION:

Mucormycosis is an opportunistic fulminant fungal infection caused by Rhizopus sp., which most commonly affects nose, paranasal sinuses and cerebral tissues. Rhizopus along with mucor and lichtheimia account for about 70-80% of all cases of mucormycosis. These fungi have the tendency to erode and invade small blood vessels and lead to thrombosis, ischemia and tissue necrosis^[1]. It is known to occur in patients with compromised systemic health as in diabetes mellitus, leukemia, prolonged corticosteroid

therapy, chronic renal failure, antineoplastic therapy, immunosuppressive therapy, deferoxamine therapy, protein calorie malnutrition owing to the impaired immunity in these patients^[2]. Mucormycosis is categorized into rhinocerebral (most common form), pulmonary, cutaneous, gastrointestinal and disseminated. Rhinocerebral form can be further subdivided depending on the tissues affected as- rhino-nasal or rhino-maxillary, rhino-orbital, rhino-cerebro-orbital^[3].

Diagnosis is based on the history, clinical presentation and investigations. Fungal culture is done

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Figure 1: Intraoral clinical presentation showing enlarged gingiva and palatal changes in the region of 16, 17.

to visualize the causative organism and the gold standard in radiological evaluation is Magnetic Resonance Imaging (MRI), followed by Computed Tomography (CT) scan^[4].

In the post COVID era, there has been a surge of cases of mucormycosis which was a seldom occurrence earlier^[5]. As Dental practitioners, we encountered a myriad of cases and evaluated them for the signs and symptoms and conducted investigations, in this case, Cone Beam Computed Tomography (CBCT). The recent development of diagnostic criteria based on CBCT findings aids in the diagnostic process.

We present a case of Mucormycosis affecting maxilla in a diabetic patient with a history of COVID-19.

A 54 year old male patient reported to the department of Oral Medicine and Radiology at Sri Aurobindo College of Dentistry, with the chief complaint of pain in the right side of his jaw since 7 days. The patient was apparently alright a week ago when he started experiencing pain in the right quadrant of maxilla which was dull, gnawing and continuous in nature which aggravated on chewing. The patient's medical history was significant for a recent COVID-19 infection 3 months ago accompanied by hospitalisation in the ICU for 10 days. Corticosteroids along with antiviral drugs were administered to the patient, after which he was discharged. His medical history revealed that he had diabetes for 4 years and

was under medication. His foregoing reports were suggestive of his blood sugar levels being consistently $>140 \text{ mg/dl}$.

On extra oral examination, there was no gross facial asymmetry, no pain or tenderness on palpation. The patient was evaluated for any sinus abnormality through palpation which yielded a negative result. On intra oral examination, swelling on the marginal and attached gingiva was observed which was non suppurative, fibrous and non tender on palpation and gentle probing [Figure 1]. On further examination, there was a diffuse slight tumescence on the right posterior part of hard palate corresponding to the teeth 16 and 17 with mild tenderness on palpation and no pus discharge. The teeth in the first quadrant had no mobility, but examination of maxilla revealed the presence of segmental mobility in the first quadrant.

Based on the history and clinical findings, a provisional diagnosis of mucormycosis of the maxilla was made and differentials included osteomyelitis, chronic granulomatous infection, and deep fungal infections.

A CBCT scan was advised and it indicated loss of cortical plate in the region of the first quadrant. On further perusal, the scan revealed an isodensity in the lumen of right and left maxillary sinuses suggestive of sinusitis. A radiographic diagnosis of Mucormycosis involving maxillary arch and invasive fungal sinusitis was made [Figure 2,3 & 4].

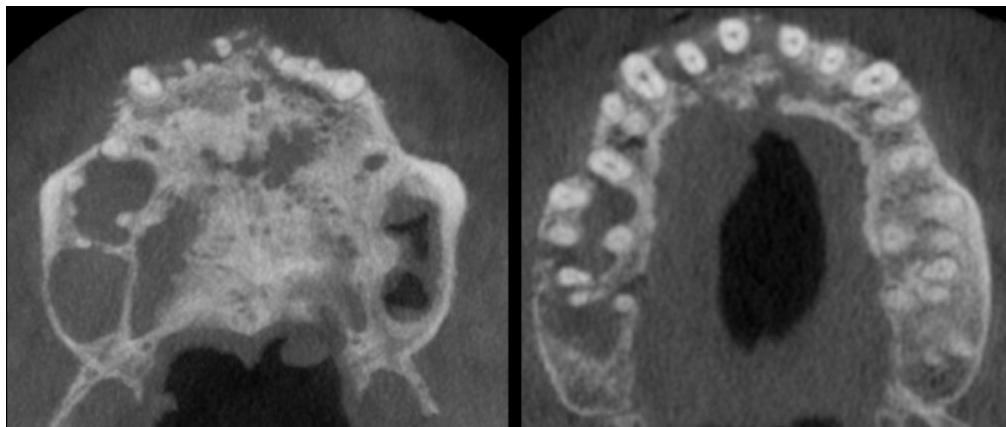


Figure 2: Shows an axial section of CBCT which depicts the loss of buccal cortical plate along with osteopenia in the region of 15, 16, 17.

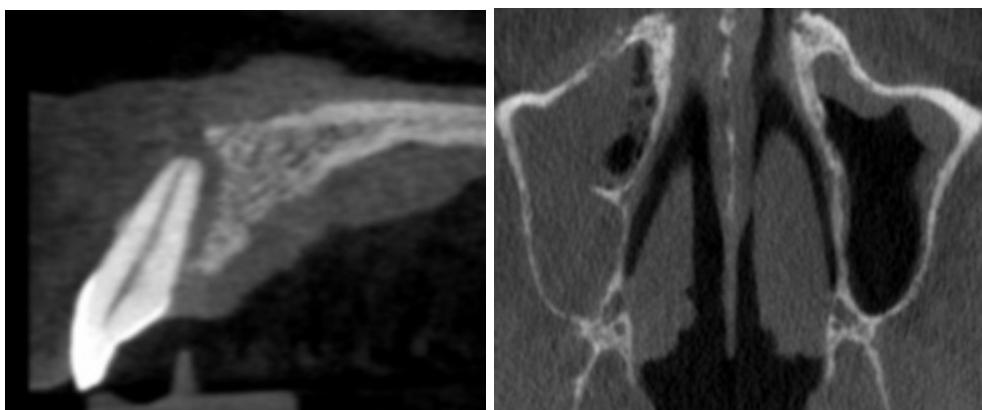


Figure 3: Sagittal section of the scan gives an insight of the bone destruction.

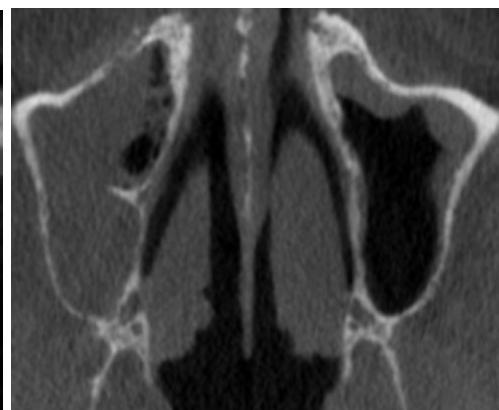


Figure 4: Axial section of the scan shows the isodensity or soft tissue hypoattenuation in the lumen of the maxillary sinus.



Figure 5: Post operative picture showing the sutured area of edentulous space



Figure 6: Post operative OPG.

Diagnostic Criteria for Rhinomaxillary mucormycosis:
[6]

- A. Involvement of at least one maxillary sinus
- B. Osteolytic changes in maxillary alveolar bone with or without involvement of other mentioned bones
- C. Presence or absence of dental findings

DISCUSSION:

Mucormycosis is an opportunistic, angioinvasive and deep fungal infection affecting the nasal cavity, paranasal sinuses, involving eye and ultimately the brain. It most commonly affects immunocompromised patients but healthy patients are not an exception. Infection arises after inhalation

through the nose and hence affects the nasal cavity and then spreads to the surrounding tissues^[1].

In 1855, Kurchenmeister described a case of Mucormycosis in a patient of neoplastic lung on the basis of its histoplasmology which was probably the first authentic human case.

In 1876, Furbringer described pulmonary mucormycosis for the first time which was caused by Absidia. The first case in humans was reported in 1885 by Paultauf^[2]. The first publication was done by Gregory et al, of the first observation of rhino-orbital cerebral mucormycosis in 1943. The first report was by Harris in 1955 of the first known survivor^[4].

According to WHO, the incidence rate of mucormycosis globally varies from 0.005 to 1.7 per million population in 2020^[7]. A 10-year study from Tamil Nadu showed an annual incidence of 18.4 cases per year during 2005–2015^[8]. Another study from Tamil Nadu reported 9.5 cases per year during 2015–2019^[8]. A multicentre study across India reported 465 cases from 12 centres over 21 months; the study reported an annual incidence of 22 cases per year, and an average of 38.8 cases for each participating centre^[8]. Chakrabarti et al showed an increasing trend of mucormycosis, with an annual incidence of 12.9 cases per year during 1990–1999, 35.6 cases per year during 2000–2004, and 50 cases per year during 2006–2007. The overall numbers increased from 25 cases per year (1990–2007) to 89 cases per year (2013–2015)^[9]. Though invasive aspergillosis is given importance among invasive mould infections in intensive-care units (ICUs), a multicentre study in Indian ICUs reported mucormycosis in a considerable (14%) number of patients^[9]. Without population-based estimates, it is difficult to determine the exact incidence and prevalence of mucormycosis in the Indian population. The computational-model-based method estimated a prevalence of 14 cases per 100,000 individuals in India. In India, prevalence of mucormycosis is about 80 times higher than the prevalence in developed countries^[7].

In India, Diabetes mellitus has been the most common risk factor linked with mucormycosis^[9]. Other causes include haematological malignancy and chemotherapy, haematopoietic stem cells, and solid-organ transplant recipients on immunosuppressive therapy, with iron overload, on peritoneal dialysis, extensive skin injury, human immunodeficiency virus (HIV) infection, and voriconazole therapy^[10].

Along with these, COVID-19 disease has a propensity to cause extensive pulmonary disease and subsequent alveolo-interstitial pathology. This by itself may predispose to invasive fungal infections of the

airways including the sinuses and the lungs. Furthermore, there is an alteration of the innate immunity due to COVID-19-associated immune dysregulation characterized by decreased T cells, including CD4 and CD8 cells^[11]. Other factors like steroid administration, immunomodulating drugs like tocilizumab, and high doses of Vitamin C, oxygen therapy, and prolonged hospitalization predispose the development of mucormycosis.

Initial clinical assessment calls for an investigation; MRI being the modality of choice to evaluate the extent of disease and prognostication. Next in line being CT although it has been observed that CBCT is one of the most underrated imaging modalities in the early diagnosis of mucormycosis as evidenced by the paucity of literature. As of late, CBCT has been considered as the examination of choice in various instances, since it gives high resolution imaging, diagnostic consistency and risk benefit assessment^[6].

In the case mentioned in this report, we evaluated the patient through proper history, assessing the clinical signs and symptoms, subjecting the patient to available modality i.e. CBCT and applying the diagnostic criteria based on CBCT findings to formulate a diagnosis.

The clinical findings included gingival enlargement, diffuse swelling involving hard palate and segmental mobility. In severe cases, a necrosed ulcerative area forms over the palate owing to the thrombosis which eventually causes exposure of the non-vital bone^[10].

CBCT images show loss of cortical bone along with obliteration of right and left maxillary sinus suggestive of invasion of sinus lumen.

In cases where the infection has spread through the other paranasal sinuses, ocular involvement takes place through the thin lamina papyracea of the ethmoid bone into the orbit resulting in rhino-sino-orbital mucormycosis^[10].

The treatment for mucormycosis infections involves the utilization of Amphotericin B, which is the initial FDA-approved drug for this purpose. Amphotericin B is available in different lipid formulations, including liposomal preparation, lipid complex, and colloidal dispersion. These formulations enhance the effectiveness of the drug and improve its administration to the affected areas, thereby assisting in the treatment of mucormycosis infections, administered in the dose of 10 mg/kg/day.^[12] A dosage of 5mg/kg/day is recommended in COVID-19 associated Mucormycosis. An oral suspension of Posaconazole 800mg/24hrs in 2 divided doses is advised for 12-13 weeks^[13].

Necrosis and thrombosis occurring during mucormycosis can lead to inadequate delivery of antifungal medications, making it crucial to consider the removal of affected tissue as an essential aspect of care for complete eradication of the infection. It is important to acknowledge that predicting surgical outcomes in mucormycosis cases is challenging due to biases in patient selection. Reports have indicated that surgical management in patients with rhino-orbito-cerebral mucormycosis yields better results compared to non-surgical treatment, enabling local control of the infection.

CONCLUSION:

Early detection and aggressive management are paramount in eradicating mucormycosis. Thorough clinical assessment, meticulous patient history, and comprehensive investigations are prerequisites for accurate diagnosis. In settings with limited resources, CBCT serves as an invaluable resource for visualizing radiographic images. This case report underscores the significance of astute clinical and radiographic examination in facilitating an early and expeditious diagnosis of mucormycosis. By emphasizing the pivotal role of these diagnostic approaches, healthcare providers can augment their capacity to promptly identify and treat mucormycosis, ultimately leading to improved patient outcomes.

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Conflicts of interest

There are no conflicts of interest.

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Case Report

Comamonas Testosteroni Emerging Gastrointestinal Pathogen

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ABSTRACT:

Comamonas testosteroni newly emerging microorganism previously known as *Pseudomonas testosteroni* is common environmental bacterium that is not known to be a part of the human commensal organism. Since its identification as a human pathogen in 1987, numerous reports have drizzled in, implicating this organism for various infections. *Comamonas testosteroni* are rare isolates in microbiology laboratories and have been infrequently reported as an infectious agent in routine clinical practice. *Comamonas testosteroni* has been rarely observed as an infectious agent in clinical practice. *Comamonas testosteroni* is rarely recognized as a human pathogen. Most of the reported cases are bloodstream infections. We report this pathogen from the stool of an immunocompromised 48-year-old male. The aim of this case report is to alert clinicians and laboratory physicians for the potential diagnosis and clinical approach of gastrointestinal infections caused by this organism.

KEYWORDS: *comamonas testosterone*; gram negative organism; gastrointestinal.

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INTRODUCTION:

Comamonas testosteroni belong to the genus Comamonas, family Comamonadaceae, which are betaproteobacteria in the pseudomonas rRNA homology group III[1]. *Comamonas testosteroni* is aerobic, gram-negative non-fermenting bacterium. They were first discovered in 1894, and since then, 24 species have been characterized. Bacterial species, including *Ralstonia spp*, *Ochrobactrum spp*, *Pseudomonas aeruginosa*, *Sphingomonas paucimobilis*, and *Brevundimonas spp*, all belong to this group^[2,3,4,5,6].

The natural habitat of these bacteria is soil, wastewater/sludge, fresh water such as ponds and rivers and the animal intestinal flora. They have also been isolated from the hospital environment and clinical samples, such as urine, pus, blood, tissue,

stool, and respiratory secretions of cystic fibrosis patients^[7]. *Comamonas testosteroni* is thought to be of low virulence. They have, however, caused infections, including serious infection such as septicemia or endocarditis in immunocompetent hosts. Herewith, we present a case of *Comamonas testosteroni* associated from stool.

CASE REPORT:

A 46 years old chronic smoker & tobacco chewer male from urban area was admitted to tertiary care hospital for chronic alcoholic liver disease with acute chronic pancreatitis due to recent alcohol intake. The chief complaint was high grade fever since 5-6 days, difficulty in breathing since last 1-2 hrs, diarrhea & abdominal pain since 1-2 days.

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Laboratory Investigation:

Routine examination showed patient blood pressure was 160/80 mmHG, pulse rate 160/min. Central nervous system, cardiovascular system appeared normal. Abdominal examination revealed tenderness in left epigastria & left hypochondria. Other relevant laboratory finding suggested hemoglobin 9 g/dl, decreased white blood cell count 5,240 mm³ (neutrophil 84%, lymphocyte 11%), platelet count within normal limit. The serological tests for human immunodeficiency virus, hepatitis B virus surface antigen, and hepatitis C virus were all non-reactive. CRP was dramatically raised to 223.6 mg/L. Stool & Blood culture was sent to the microbiology laboratory.

Radiological finding:

Ultrasonography revealed cholecystitis and small amount of fluid noted in peripancreatic area. Patient was given empirical therapy with fluid and electrolyte replacement and kept on piperacillin-tazobactam & Vancomycin as empirical antibiotic treatment.

Laboratory workup:

Blood Culture was kept in BacT/ALERT/3D automated blood culture system by Biomerieux for 7 days of incubation. Stool culture streaking was done on MacConkey agar, 5% sheep blood agar & XLD agar plate. Other relevant hematological investigation were also performed.

Findings:

In stool culture, after overnight incubation on MacConkey agar, colonies were non-lactose fermenting, small translucent, smooth & without pigment (Figure 1). Sheep blood agar plate showed non-hemolytic, non-pigmented colonies similar as on MacConkey agar. Colonies were Oxidase positive, Catalase positive, Gram negative and motile in hanging drop preparation (Figure 2). They were than processed for identification and sensitivity by Vitek-2 compact by Biomerieux using GN and N406 for identification and sensitivity respectively. It was identified as *Comamonas testosteroni* with 99% probability. It was found sensitive to Gentamicin, Amikacin, Imipenem, Meropenem, Piperacillin-Tazobactum, Cefoperazone-sulbactum, Cefepime and Minocycline. And it was found resistant to Ofloxacin,

Colistin, Ceftazidime, Aztreonam, Ciprofloxacin, Levofloxacin and Trimethoprim-Sulfamethoxazole. Blood culture of the same patient remained sterile for 7 days of incubation. Therapy was changed to Gentamicin 4 mg/kg/dose daily and imipenem 25 mg/kg/dose 8 hourly for 10 days. The patient responded well to antibiotic therapy.



Figure 1 : Colonies of *Comamonas testosteroni* on MacConkey agar.



Figure 2 : Colonies of *Comamonas testosteroni* on Sheep blood agar.

DISCUSSION:

Comamonas testosteroni are ubiquitously found in nature and have a global distribution. Intra-abdominal infections are the commonest infections reported with this organism^[8]. Most of the previously reported cases were immunocompromised due to different condition. Our case was also immunocompromised due to alcoholic chronic liver disease leading to chronic pancreatitis. *Comamonas testosteroni* is the most common species among its various species causing human infection and majority

of the patients were survived. Intra-abdominal infections are the most common infections caused by *Comamonas testosterone*, especially in individuals with predisposing conditions^[9]. Bacterial translocation from gastrointestinal tract seems to play an important role in the pathogenesis caused by *Comamonas* species. *Comamonas* species are inherent and are able to survive in environment which makes it suitable candidate for chronic and mild infection^[9]. In our case there was no specific source of infection identified. Immunocompromised status of the patient plays a major role in producing infection by this organism. One incidence of infection by *Comamonas testosterone* has been reported in stool culture in elderly women with colostomy. The aim of this case report was to alert clinicians and laboratory physicians about the potential diagnosis and clinical approach of gastrointestinal infections caused by this organism.

CONCLUSION:

We reported a rare case of *Comamonas testosterone* associated with stool. This case highlights a thorough clinical and laboratory work-up that is necessary for a positive outcome.

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Conflicts of interest

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